New Anticancer Drugs: Targeting Tubulin and Signal Transduction Pathways

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Abstract

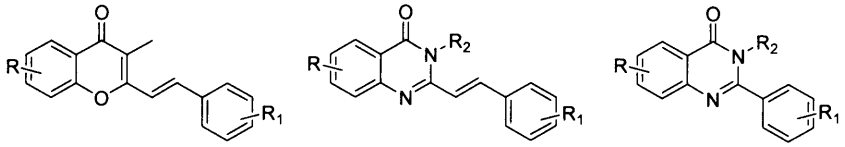
The main aim of the study described in this thesis is the development of new anticancer agents. The first chapter is a general introduction to cancer, and the development of chemotherapy anticancer agents during the course of the years.

The following four chapters briefly introduce the biological targets in the authors study.

Chapter Two describes a general introduction to tubulin and microtubules as anticancer targets. A discussion of those compounds most relevant to this thesis is provided. Chapter Three describes Signal Transducers and Activator of Transcription 3 (STAT3) proteins, their role in cancer and the advances in the search of anticancer agent inhibitors of the STAT3 signalling pathway. Chapter Four focuses on the src homology 2 (SH2) domain containing tyrosine phosphatases SHP-2, a protein-tyrosine phosphatase implicated in pathogenesis of cancer and other human diseases. A brief discussion of the SHP-2 inhibitors is provided. Chapter Five describes the role of proteins Aurora kinases in cancer, promising targets for anticancer drug development, and the advances in the search of their inhibitors targeting the kinase activity at the ATP binding site.

The following chapters (6-11) describe the authors own findings.

Chapter six focuses on the design and synthesis and biological evaluation of novel styrylchromones, styrylquinazolones, and quinazolones as inhibitors of tubulin polymerization.



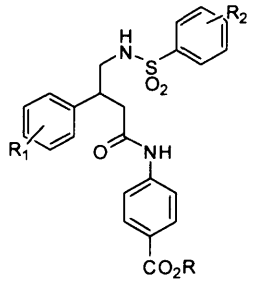
Styrylchromones *Styrylquinazolones* *Quinazolones*

Two series of isomeric styrylchromones were initially synthesized in order to establish the methoxy substitution pattern on the A ring favorable for optimal activity. The structure activity relationship on the B ring is also reported. Next, our strategy focused on identifying a chromone core replacement with improved potency. We directed our chemical efforts toward the synthesis of novel styrylquinazoline analogs. The quinazoline core would also provide easy access to the preparation of diverse sets of *N*-substituted derivatives (methyl and ethyl derivatives).

Finally, a novel series of quinazolines were synthesized as conformationally-restricted analogs of chalcones. SAR was conducted around the quinazoline *spacer* between the aryl rings and systematically investigating the substituent effect in the B ring.

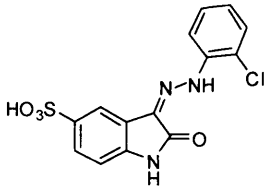
Among the synthesized compounds we selected those analogues showing significant cytotoxicity (generally defined as IC_{50} value < 1.5 μM), and evaluated for activity *in vitro* tubulin polymerization inhibition assay.

Chapter Seven focused on the identification of novel inhibitors of STAT3 dimerization. Computational analyses led us to the development of a T-shape model of molecules that can occupy the pTyr-binding pocket of STAT3 SH2 domain. The conjugate addition of nitromethane to a series of amides and the reduction of the nitro group were combined to give an easy route to the target T-shape molecules in a combinatorial fashion. The methodology was also extended to amides activated by a nitro group. We observed a dramatic change in the course of the reaction, which afforded a mixture of unexpected and unknown products, that each possessed an additional methylene group. A brief study into the mechanism was also conducted.

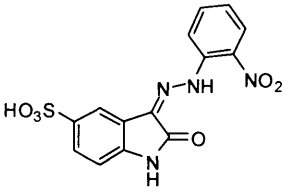


Scaffold of T-shape molecules

Chapter Eight, Nine and Ten focuss on the development of Aurora kinases and SHP-2 inhibitors. Oxindole derivatives **HL10581** and **NSC117199** emerged as lead compounds from a high throughput screen for Aurora-A and SHP-2, respectively.



HL10581, Aurora-A, IC_{50} 1-5 μM



NSC117199, SHP-2, IC_{50} 47 μM

Chapter Eight describes the synthesis of several derivatives of **HL10581** and **NSC117199**, directed to exploration of SAR around the oxindole moiety to determine the structural features that are responsible for the activity. Chapters nine and ten report the biological evaluation of oxindole derivatives as inhibitors SHP-2 and Aurora kinases, respectively

Acknowledgements

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Contents

Abbreviations

ADME	Absorption, Distribution, Metabolism, Excretion
ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
BOC	*tert*-Butyloxycarbonyl
CDK2	Cyclin Dependent Kinase2
CDPs	Cysteine-Dependent Phosphatases
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DEC	Dichloroethane
DMAP	4-*N,N*-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DPP	Diaminedichloroplatinum
EGFRs	Epidermal Growth Factors Receptors
EGTA	Ethyleneglycol-*bis*-(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid
EMSA	Electrophoretic Mobility Shift Assay
FDA	Food and Drug Administration
GQ-ODNs	G-quartet-Oligodeoxynucleotides
GR	Growth Factor
GTP	Guanosine Triphosphate
GTPases	Guanosine Triphosphate Hydrolases
FRET	Fluorescence Resonance Energy Transfer
HMPA	Hexamethylphosphoramide
HOBt	Hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
HTS	High Throughput Screening
ITP	Inhibition of Tubulin Polymerization
JAKs	Janus Kinases
JMML	Juvenile Myelomonocytic Leukemia
MAPs	Microtubule Associated Proteins
Mes	2-(morpholino)ethanesulfonic acid

MOM	Methoxymethyl
mRNA	messenger Ribonucleic Acid
MTOCs	Microtubule Organizing Centres
NBS	N-Bromosuccinimide
NCI	National Cancer Institute
NMR	Nuclear Magnetic Resonance Spectroscopy
nOe	nuclear Overhauser effect
Np	Naphthyl
NS	Noonan Syndrome
ODN	Oligodeoxynucleotides
PDB	Protein Data Base
PIAS	Proteins Inhibitors of Activated STATs
$POCl_3$	Phosphorooxichloride
PTPs	Protein Tyrosine Phosphatases
PTKs	Protein Tyrosine kinases
SAR	Structures Activity Relatioship
SMPIIs	Small Molecule Inhibitors of Protein-Protein Interactions
SOCS	Suppressor of Cytokines Signaling
STATs	Signal Tranducers and Activators of Transcriptions
THF	Tetrahydrofuran
UICC	International Union against Cancer
VDAs	Vascular Disrupting Agents
VEGFs	Vascular Endothelial Growth Factors
VEGFRs	Vascular Endothelial Growth Factor Receptors
WHO	World Health Organization

Chapter 1

1.0 Introduction

1.1 What is cancer?

The origin of the word *cancer* is credited to the Greek physician Hippocrates (460-370 B.C.), considered the "Father of Medicine." Hippocrates used the terms *carcinos* and *carcinoma* to describe non-ulcer forming and ulcer-forming tumors. In Greek these words refer to a crab, most likely applied to the disease because the finger-like spreading projections from a cancer called to mind the shape of a crab. Carcinoma (cancer arising from epithelial cells) is the most common type of cancer. Nowadays, cancer is defined a group of diseases characterized by uncontrolled cell division and uncontrolled cell growth. The resulting mass, or tumor, can invade other tissues, either by direct growth into adjacent tissue (invasion) or by migration of cells to distant sites (metastasis). If the spread of abnormal cells is not controlled, it can result in death. This unregulated growth is caused by a series of acquired or inherited mutations to DNA within cells, damaging genetic information that define the cell functions and removing normal control of cell division.

1.2 Social impact of cancer

Cancer is a disease of worldwide importance because it is a major killer throughout human history. It is not a surprise that from the dawn of history doctors have written about cancer. Some of the earliest evidence of cancer is found among fossilized bones tumor, human mummies in ancient Egypt, and ancient manuscripts. Early in the 20th century, the only curable cancers were small and localized enough to be completely removed by surgery. Later, radiation was used after surgery to control small tumor growths that were not surgically removed. Finally, chemotherapy was added to destroy small tumor growths that had spread beyond the reach of the surgeon and radiotherapist.

Cancer is still a growing problem and represents a major health threat in most parts of the world. Cancer killed 6.7 million people around the world in 2002 and this figure is expected to rise to 10.3 million in 2020. Total cancer has been rising steadily in the USA and EU up to the late 1980s. At the beginning of 2000, tangible progress had been made, but a relatively little decrease of cancer mortality had been achieved.[1] Better prevention, screening and early diagnosis change in lifestyle (i.e. giving up tobacco smoking) have played a key part in leveling out the incidence and mortality rate for some cancers.[2,3,4] Despite the expanding knowledge[5] of cancer and the number of advanced available treatments such as surgery, chemotherapy and radiations, there is still a major gap between the efforts of cancer research and the practical results achieved. In the 1970s the possibilities of "conquering cancer" and

"finding the cure" were surrounded by high expectations because of the advances in cancer research and social impact of a disease like cancer, especially in view of what had been achieved for other common diseases in the past. For instance, we can recall the enormous impact of the advent of antibiotics on the industrialized countries or the merit of polio vaccine for disappearance of poliomyelitis. Even if so relatively little seems to be achieved, we have to be aware that what we are facing is not a simple disease but, by definition, an array of diseases, each one has its own specificity. Even within the same tumor in the same patient not all the cells are similar and they present thousands of random mutations. These variables can affect the response to treatments.

Over the last four decades cancer research has also benefited of a worldwide cooperation of many cancer-related organizations such as the International Union against Cancer (UICC), the World Health Organization (WHO) and the American Cancer Society, which has improved the capacity building for cancer organizations and the information exchange and delivery. Substantial investments have resulted in appreciable progress in our knowledge and understanding of the mechanisms involved in tumor pathogenesis and progression. In the past 5 years the completion of the human genome project and the advances of molecular biology have had a huge impact on cancer research and offered an enormous number of novel potential therapeutic targets.[6,7,8] Owing to a lack of understanding of the molecular mechanisms that drive oncogenesis, up to a few years ago the only mechanism available was cytotoxicity or inhibition of cell proliferation. The current tendency in anticancer drug discovery is based on the concept that more selective and target orientated therapies can be developed by identifying the biological differences between normal and tumor cells. Hopefully the new smart target oriented therapies will change the life perspectives of cancer patients granting a better quality life.[6,7,8]

1.3 Traditional treatments of cancer and chemotherapy

The major types of treatment for cancer are surgery, radiation, and chemotherapy.

Surgery is one of the main treatments for cancer. The Roman doctor Gallien first wrote about surgery as cancer treatment in the 2nd century. Over the centuries surgical treatments for cancer went through a slow process of development and surgery was initially very primitive with many complications. The era of surgery began with the discovery of anesthesia in 1846 and since then several surgical techniques have been developed and improved leading to the modern cancer surgery.

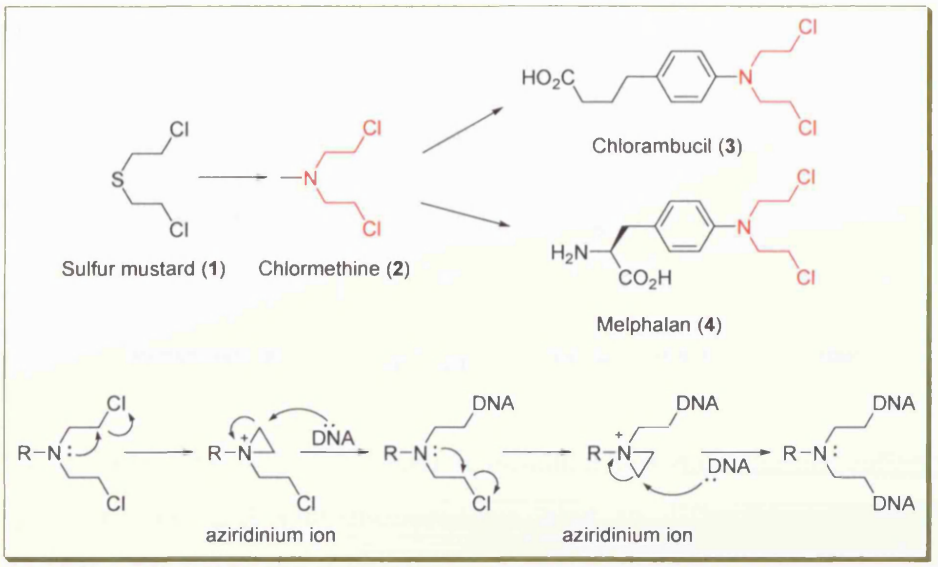
At the beginning of the twentieth century radiation became an important treatment modality from which cancer patients could benefit. Radiation therapy began with radium and with relatively low-voltage diagnostic machines. Although the current methods and the machines for delivery of radiation therapy have dramatically improved allowing destruction of malignant tumors with great precision, nowadays radiation therapy is still limited by the severe side effects and a limited capacity to discriminate between healthy and tumour cells. Moreover, both radiation and surgery are not curative in cases of advanced metastatic diseases.

For the majority of the twentieth century, cancer research and drug discovery programs have focused on the identification and development of chemotherapeutic agents to treat and fight human cancers. The early stage of chemotherapy history has been mainly characterized by accidental discovery or random screening of natural or synthetic compounds using cell cytotoxicity assays.[9] Unfortunately none of these compounds were particularly specific to the cancer cells. The conventional chemotherapy, also called cytotoxic therapy, has been based on the theory that rapidly proliferating and dividing cells are more sensitive to cytotoxic agents than normal cells. Most of these drugs act by targeting DNA, tubulin or topoisomemerases and interfere with cell division. This has been the case of nitrogen mustard, platinum based compounds, taxanes, Vinca alkaloids, Camptothecins.

The era of chemotherapy began in the 1940s with the first uses of nitrogen mustards and folic acid antagonist drugs. During World War, the U.S Army was studying a number of agents related to mustard gas in order to develop more effective agents and protective measures. In the course of that work, a compound called *nitrogen mustard* or Chlormethine (**2**) was studied and found to have substantial activity against lymphoma (Figure 1).[10,11] Nitrogen mustard is an example in which an initial astute clinical observation and medicinal chemistry have together led to agents such as Chloambucil (**3**), Melphalan (**4**) still in clinical use today.[12]

Chlormethine (**2**) is a derivative of sulfur mustard gas (**1**) which was found to lower the white-blood-cell count but too toxic to be used as a therapeutic agent. The toxicity sulfur mustard gas (**1**) had been hypothesized to be related to its reactivity towards electron-rich groups such as the phosphates in nucleic acids under conditions present in the cells. Based on this theory Gilman designed and synthesized some less electrophilic derivatives by replacing the sulfur with a substituted nitrogen leading to new derivatives, Chlormethine (**2**) Chloambucil (**3**), Mephalan (**4**), having a decreased toxicity to normal cells.[10,11] Due their ability to add alkyl groups to DNA under conditions present in cells, the sulfur mustard gas derivatives are generally referred to as "alkylating agents.

Figure 1. Structures of mustards and their chemical reaction with DNA.

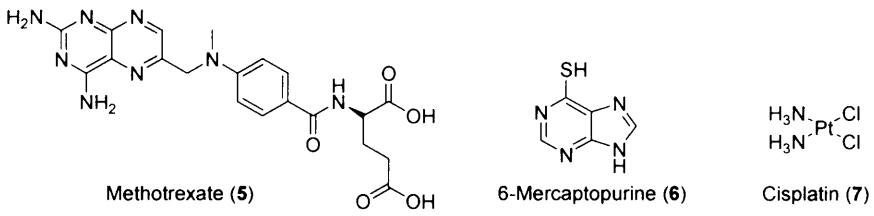


With the discovery of nitrogen mustard, DNA became an unique target for cancer research. Design and development of analogues of metabolites needed by DNA to replicate or a cell to divide became an appealing strategy for anticancer therapy. For instance, Methotrexate (**5**), also known as anti-metabolite, was synthesized as analogue of folic acid, which is required for DNA metabolism (Figure 2). Methotrexate (**5**) acts as an antagonist to folic acid blocking a critical chemical reaction in the synthesis of thymidine, needed for DNA replication. A successful sub-class of anti-metabolites is the purine derivatives [e.g 6-Mercaptopurine (**6**), Purinethol®], therapeutic agents used to treat acute lymphatic leukemia (Figure 2). Purinethol masquerades as purine and becomes a building block of DNA preventing purines to become incorporated into DNA during cell division, stopping the normal development and proliferation.

Cisplatin (**7**) —"the penicillin of the cancer drugs"— represents another example in which an accidental discovery and chemistry led together to the development of a clinically useful anticancer agent (Figure 2). In the 1960s Rosenberg and co-workers observed that electrolysis products from a platinum electrode inhibited mitosis in *Escherichia coli* bacteria. A product of the reaction between the platinum electrodes a constituent of the culture medium [later determined to be cis-diaminedichloroplatinum(II) (DDP) or cisplatin] was found to be responsible for the inhibition. Cisplatin, approved for clinical use by the United States Food and Drug Administration (FDA) in 1978, has been one of the most widely prescribed and one of the most effective treatments for many types of cancer such as testicular, ovarian, bladder,

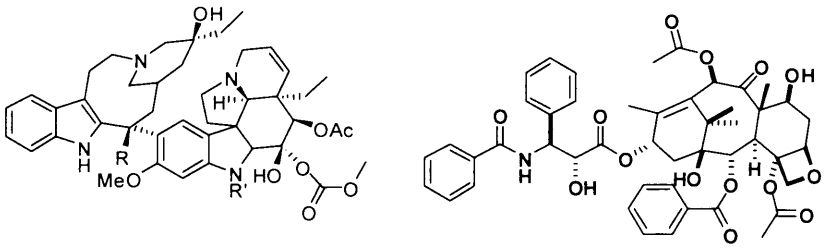
and lung and stomach cancer. It is believed to act by cross-linking DNA and interfering with cell's repair mechanism leading to cell's death.

Figure 2. Structures of Methotrexate (**5**), 6-Mercaptopurine (**6**), and Cis-platin (**7**).



Besides DNA, microtubules and tubulin have represented important pharmaceutical targets. A large group of natural and synthetic products bind to different sites on tubulin or microtubules. By suppressing the microtubules dynamic, they block the mitosis and inhibit cell proliferation. Many of these drugs (also known as antimitotic agents) are well established treatments of various cancer. At the present the clinical use of agents targeted at tubulin is restricted to the vinca alkaloids [e.g Vincristine (**8**) and Vinblastine (**9**)],[13] taxanes [e.g. Paclitxel (**10**)][14] (Figure 3). Both classes of compounds are based on complex and large natural products, emerged from the screening of extract of plants for cytotoxicity against cancer cell line.

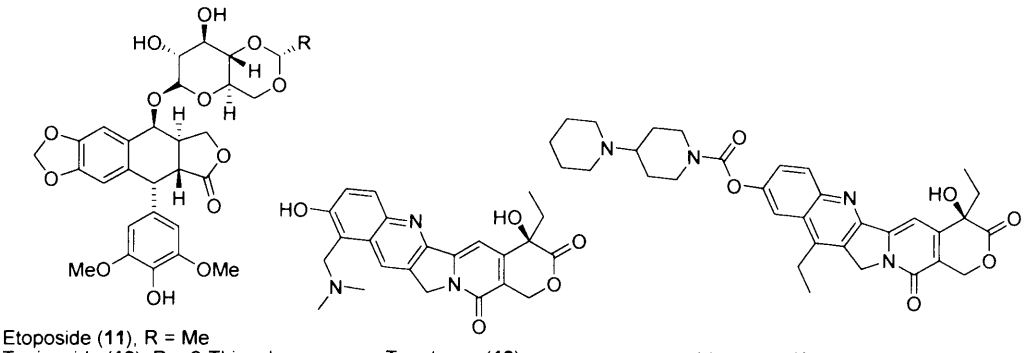
Figure 3. Structures of Vincristine (**8**), and Vinblastine (**9**), and Paclitaxel (**10**).



Many of the clinically effective drugs have been found to show their cytotoxicity through the stabilization of the topoisomerase (I or II)-DNA complex (also referred to as cleavage complex), forming ternary complexes (Figure 4). DNA topoisomerases are essential for DNA replication, transcription, chromosome segregation, and DNA recombination. Examples of topoisomerases inhibitors include Etoposide (**11**) (topoisomerase II), Teniposide (**12**) (topoisomerase II), Camptothecin derivatives such as Topotecan (**13**) (topoisomerase I),[15] and Irinotecan (**14**) (topoisomerase I).[15] The stability of the ternary complex and the strenth of the

binding result in the potency of the drugs[16] but not in the selectivity of the agents that target this process. In fact, similar drug-target molecular interaction occurs in diseased as well as normal cells resulting in a very high toxicity.

Figure 4. Topoisomerase I and II poisons.



As mentioned before, the current cancer research is moving from purely cytotoxic drugs (otherwise referred as to "hard" drugs) to molecular targeted therapies based on "soft" drugs[7,17] acting specifically on tumor cells. Despite the new trend of cancer research, the importance of conventional established cytotoxics cannot be denied and they remain extensively used in the clinic to fight the most aggressive solid tumors. Significant efforts have been done in order to improve the tumor cell selectivity and the therapeutic index of the conventional cytotoxic agents and overcome drug resistance. DNA is still considered as an appealing target for the development of selective anti cancer strategies.[18] Further investigation of the mode of action has shown that topoisomerase poisons exhibit a well-defined preference for a given DNA sequence. The toxic effects of the individual drugs might be minimized by increasing their sequence selectivity. Among the possible approaches, an enhanced selectivity for tumor cells could be achieved by linking a cytotoxic drug to a DNA-recognition moiety. In this manner, the hybrid molecule would combine the DNA-targeting and damaging properties conferring higher DNA-affinity without altering the DNA-drug interaction.

New strategies for cancer research have focused on telomerase as a novel and selective DNA anticancer target. Telomerase catalyses the synthesis of telomeric DNA, which comprises short repeat sequences at the end of chromosomes. In mature somatic cells in human tissue were found undetectable or low telomerase activity, due to the fact that their telomeres become progressively shortened with successive round of replication until they become critically short and the cell undergoes apoptosis.[19]

Around 80% of human cancers escape from this growth arrest by re-activating telomerase but at diagnosis many cancers still have very short telomeres making them very vulnerable to the inhibition of telomerase. Moreover, telomere maintenance is essential to the replication process in malignant cells and to the progression of the disease. High level of telomerase activity has been detected in tumor cells, which stabilize their telomeric ends by action of reverse transcriptase telomerases.

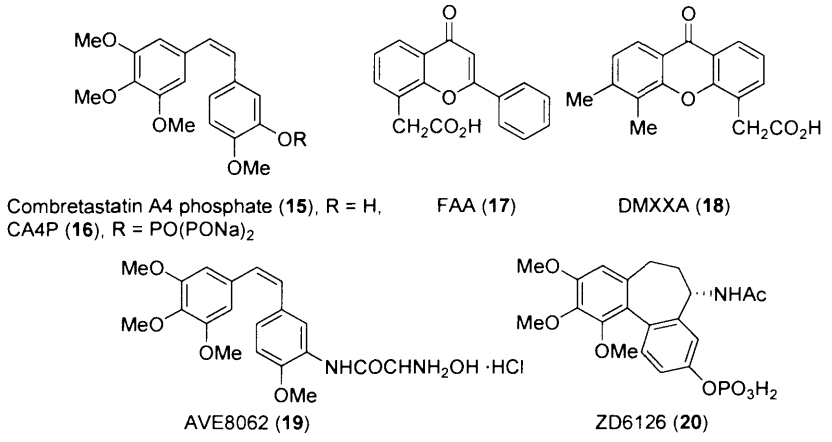
Identification of molecular inhibitors of telomerase activity might represent a useful strategy for treatment of cancer. Given the low telomerase activity expressed in normal cells, telomerase-directed drugs should not interfere with healthy somatic cells. They are not expected to damage appreciably germ like cell, which have in general longer telomerase than cancer cells. The identification of agents capable of stabilizing a particular folded conformation of telomere (called G-quadruplex)[20] which are not recognized by the template of telomerase, have been described as potential and promising approach.[21-24] Antisense oligonucleotides strategies have also been investigated.[25,26] Concerns about the possible limitations of the telomerase inhibition have been raised. They are mainly related to the eventual drug resistance cancer cells can develop.[6] Some tumours might be intrinsically resistant to telomerases based-drugs because not all the tumour types are characterized by detectable levels of enzyme activity.

New interest in tubulin-binding agents has been recently stimulated by the discovery of the vascular-damaging properties of Combretastatin A4 (**15**), a tubulin-depolymerizing agent (Figure 5). Tumor vasculature has become a valuable target for anticancer therapy[27-29] due to its critical role in the process of tumor growth and metastasis. Cancer vessels are essential to supply a growing solid tumour with oxygen and nutrients and to remove toxic waste of the cellular metabolism. In order to ensure continued growth and development tumors must generate their own network of microvessels through the process of angiogenesis.[28,30] In addition, the tumour vasculature differs from the vasculature in normal tissue.[28] These differences may open the path towards highly selective treatments of cancer.

Therapeutic vascular targeting has initially focused on the inhibition of the angiogenesis.[28,29] The anti-angiogenic strategy has been supported by the discovery of specific vascular endothelial growth factors (VEGFs) –a class of proteins responsible for the regulation of angiogenesis– and their receptors (VEGFRs).[31,32] Overexpression of VEGFs and VEGFRs in tumor cells is well documented.[32,33] Disruption of the production and expression of these factors has been considered as a valuable tool for the inhibition of tumor growth and metastasis.[34,35-37] Numerous are the drug discovery programs aimed at the development of

inhibitors of VEGFs or their receptors. Many of these agents are currently in various development stages or undergoing clinical trials.[38,39]

Figure 5. Main vascular disrupting agents (VDAs).



The alternative approach to the anti-angiogenic strategy is the targeted destruction of the established tumor vessels network.[40] The vascular disrupting agents (VDAs) cause the rapid and selective shutdown of the tumor vasculature producing tumor death from ischemia and necrosis[28] (Figure 5). At the present there two main types of small molecules VDAs: tubulin-depolymerizing agents such as Combretastatin A4 (**15**) and combretastatin derivatives (Figure 5), and other flavonoids, such as FAA (**17**) and DMXAA (**18**). Classical tubulin-binding agents colchicines and Vinca alkaloids were found to disrupt tumour vasculature at near toxic doses.[41-43] In contrast, the combretastatins [CPA4 (Oxigene) (**16**), AVE8062 (**19**) (Ajjnomoto/Aventis) and ZD6126 (**20**) (Angiogene/AstaZeneca)], undergoing clinical trials, induce very quickly vascular shutdown at doses that are less than one tenth of the maximum tolerated dose,[44-48] selectively in tumor[44,46,49] (Figure 5).

The antivascular effect of these compounds appears to derive from their tubulin binding properties. The drugs cause the microtubules to depolymerize and the endothelial cells round-up, blocking the blood flow through the tumour vascular network. This effect is mostly pronounced for agents that bind at the Colchicine binding site like Combretastatin A4.

1.4 The impact of modern biology and medicinal chemistry on cancer treatments

Prior to the genomic era chemotherapeutic agents have been discovered by chance or by inhibiting metabolic pathways crucial for cells division. The exact reverse strategies are being used by the current cancer research. A better understanding of the cellular, molecular and

genetic basis of cancer has led to the discovery of the key distinguishing features between normal and diseased cells. Modern biology has focused on understanding and studying of the molecular pathways altered in cancer aiming at translating them into therapeutic strategies. Often the mode of action of these cytotoxic agents has been elucidated after their anti-tumor activity had been established in clinical trials.

The novel target oriented therapies would allow to: a) hit the desired target reducing the effective concentration, b) react with a specific active site without interfering with other unrelated biological process increasing the therapeutic index, c) play with a wide concentration window to circumvent drug resistance.[6]

Currently the most promising investigation areas in the anticancer drug development[6,7,18,50,8] are focused on the generation of new and more specific and more effective agents that target DNA-associated process such as new cytotoxics and telomerase inhibitors, the process of angiogenesis and metastasis, and the cell signaling

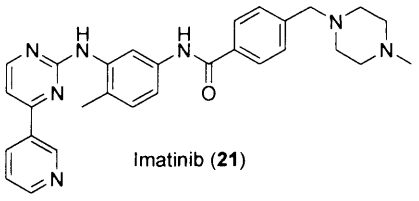
1.4.1 Cell signaling

Cell signaling is part of a complex system of communication that regulates the cell activities. Living cells are constantly exposed to a variety of signals from their micro- and macro-environments and their ability to respond to these signals is the basis of the cell functions. Some cell-to-cell communication requires direct cell-cell contact through gap junctions that connect their cytoplasm to the cytoplasm of adjacent cells allowing different ions and molecules to pass freely. Many cell signals are carried by molecules called receptor ligands (e.g. hormones, cytokine, neurotransmitters and growth factors), released by one cell and move to make contact with another cell. The specificity of signaling can be achieved and controlled if only specific cells can respond to a receptor ligand. Cells receive information from their environment through a class of protein called receptors located on the cell membrane or within the cytoplasm or cell nucleus. The formation of the ligand-receptor complex results in the cellular response to the ligand. In some cases receptor activation caused by ligand binding to a receptor is directly coupled with the cell's response. In some other cases the ligand-receptor interactions are not linked to the direct cell response. The transmission of extra-cellular signals into their intra-cellular targets is mediated by a network of interacting proteins that regulates a large number of cellular processes. The set of biochemical reactions carried out by proteins or enzymes, induced by receptor activation are called signal transduction pathways. The sequential activation of enzymes is also called a signaling cascade.

Errors in the signal processing are responsible for diseases such as cancer, autoimmunity, and diabetes. The altered signaling responses are often critical distinguishing features between normal and tumour cells. There is a general consensus that signaling molecules actively engaged in the regulation of the tumour pathogenesis and progression could be potential targets for cancer therapy.[7,18,51]

1.4.2. Targeting tyrosine kinases: Imatinib Mesylate

Protein kinases are enzymes components of signal transduction pathways, playing different roles in normal physiological cell processes, such as control of cell growth, metabolism, differentiation, and apoptosis. They comprise two major subfamilies, the protein serine/threonine kinases and protein tyrosine kinases. A kinase acts by transfering a phosphate group from ATP (adenosine 5'-triphosphate) and covalently attaching it to other proteins. The process is called phosphorylation. The interest in the tyrosine kinase pathways is due to the oncogenic role of protein kinases in cancer cells.[52] Kinases have been initially targeted for validating the clinical effectiveness of the development of signal transduction inhibitors as a new for anticancer strategy. A great enthusiasm around this research area has been further raised by the discovery of Imatinib Mesylate. Imatinib mesylate (Glivec®) (**21**) is an example of targeted therapy and the first anticancer agent that works by inhibiting a specific signaling kinase instead of non-specifically inhibiting rapidly dividing cells. It is a small molecule selective inhibitor of bcr-abl fusion protein, a tyrosine kinase enzyme (PTK), unique to leukemic cells and expressed at high level. Its tyrosine activity is essential for its ability to induce leukemia.[53,54] Glivec was not a serendipitous discovery but is the result of application of a rational approach to identify chemical leads inhibitors of a specific target. Further chemistry-based design works on the lead compound were required to transform the lead molecule into a drug. Bcr-abl kinase gained a great interest as target for the design of small molecules selective inhibitors, based on the hypothesis that the decrease of the activity of bcr-abl kinase would contribute to induce a remission of the disease and have little effects on normal cells.



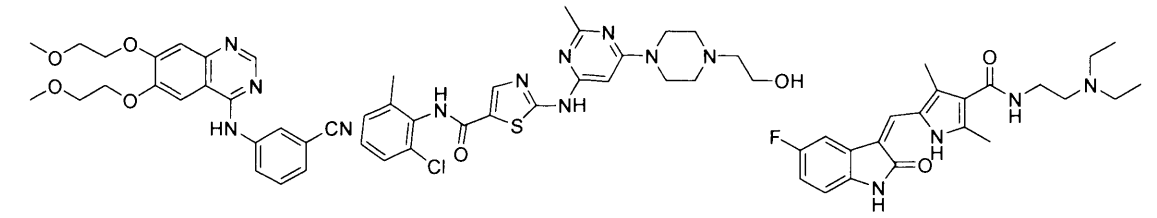
Imatinib (**21**)

In 1988 Yaish and co-workers reported a class of compounds called tyrphostins as epidermal growth factor receptor kinase inhibitors[55] providing the proof of principle that pharmacological inhibitors could target a specific tyrosine kinase. Buchdunger and co-workers demonstrated that, an Abl protein-tyrosine kinase inhibitor of the 2-

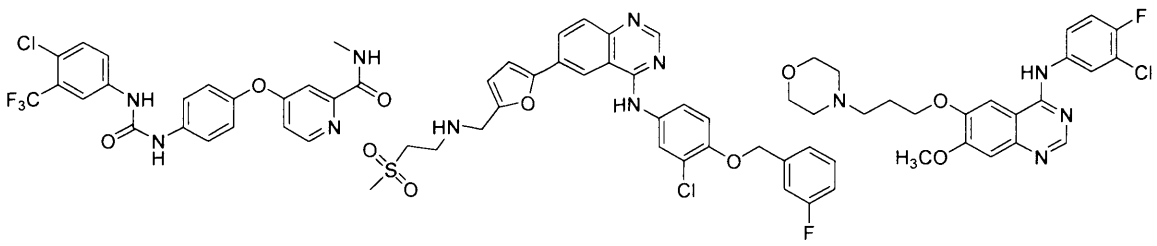
phenylaminopyrimidine family, identified from high throughput screen of chemical libraries,[56,57] induced the selective inhibition of the platelet-derived growth factor signal transduction pathways. The initial inhibitors were of low specificity and potency, but the inhibitory activity could be optimized by synthesizing and screening focused libraries of 2-phenylaminopyrimidine derivatives. Imatinib emerged as the suitable candidate for preclinical development. Further studies of the mode of action revealed that Imatinib functions as a competitive inhibitor of ATP binding.[58] Imatinib was approved by the United States Food and Drug Administration (FDA) in May 2001.

Many kinase inhibitors have completed the clinical trials and received the FDA marketing approval. The structures of all kinase inhibitors (targeting the ATP binding site) currently in use are provided in Figure 6.

Figure 6. Structures of all kinases inhibitors in use.



Erlotinib (**22**) Dasatinib (**23**) Sunitinib (**24**)



Sorafenib (**25**) Lapatinib (**26**) Gefitinib (**27**)

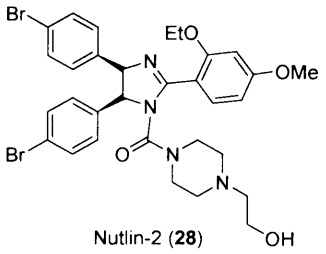
1.4.3. Protein-protein interactions as a target for anti-tumor agents

Protein-protein interactions play a central role in signal transduction pathways that regulate biological processes.[59] Inappropriate protein-protein binding can lead to diseases such cancer and diabetes. It should not be surprising that protein-protein interactions represent attractive pharmaceutical targets[60] and the identification of small molecule inhibitors of protein-protein interaction (SMPIIs) has become a promising field in drug discovery.[61] Years ago a few description of SMPIIs were reported in the literature. Unfortunately the development and design of small molecules that can modulate the protein-protein binding has been problematic, owing to issues such as the lack of well-defined binding pocket.[62,63,64,65]

Antibody (dominant negative proteins)-based antagonists, or medium-sized peptide were investigated as therapeutic agents. The prevailing medicinal chemistry and drug discovery perspective was that protein-protein interactions would be difficult to influence using small molecules.[66]

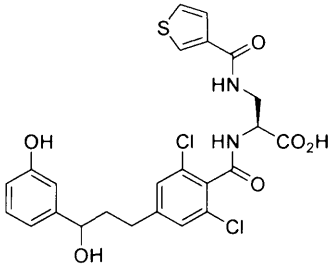
However, there have been important progresses in the field in recent years. The increasing number of publications reporting emerging classes of protein-protein interaction inhibitors and improved strategies employed for the discovery of small molecules modulators has contributed to change this view.[62,67,68,63] A recent success is the identification of Nutlin-2 (**28**), an important inhibitor of p53-MDM2 interaction. The discovery was claimed by Hoffman-La Roche Inc. in February 2004.[69]

Despite the enthusiastic expectations generated by the advances in understanding of tumour pathogenesis and progress, it has been argued that the identification of the clinical relevance of the new molecular targets not always can be translated into a strategy with clinical utility.[7] Not all the molecular targets are druggable or, in other words, it is often difficult and problematic the conversion of lead compounds into molecules with pharmacological properties, otherwise called "drug-like" molecules.[70,71] Chene concluded his review published in January 2004 on the inhibition of the p53-MDM2 interaction and the targeting of protein-protein interface[72] highlighting how the difficulties accompanying the identification of small molecule modulators of protein-protein interactions might not be overcome despite the research efforts. At this moment, only a few drugs targeting p53 had been identified. The best compounds described in the literature, even if potent, were not druggable molecules. A month later the hope became reality by the discovery of new small "smart" molecule inhibitors of p53-MDM2 interaction. These results gave new input and confidence to the search of modulators of protein-protein interactions leading to the subsequent discovery of novel protein-protein interaction modulators and validation of their targets. These compounds may act either directly – via inhibition at the protein-protein interface – or indirectly – via binding to an allosteric site and induction of conformational changes of the target protein.[61,67,73] Selected examples of small molecule protein-protein interaction inhibitors and their targets are illustated in Figure 7.

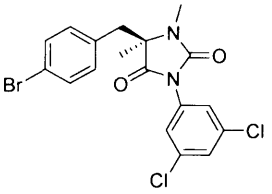


Nutlin-2 (**28**)

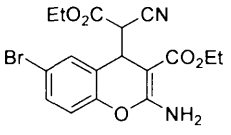
Figure 7. Selected examples of small molecule protein-protein interaction inhibitors and their targets.



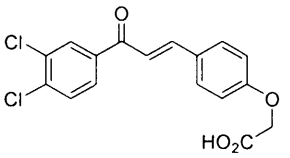
29, LFA/ICAM-1



30, LFA/ICAM-1



31, Bak-BH3/Bcl2



32, MDM/p53

1.5 Conclusion

An understanding of cancer pathogenesis at cellular, molecular and genetic level has led to the discovery of the key distinguishing features between normal and diseased cells, revealing a wide spectrum of new potential clinical targets for development of novel drug with enhanced potency and selectivity. Hopefully this information will be important in improving drug efficacy and in offering better life perspectives to cancer patients.

Chapter 2

2.0 Tubulin and microtubules as anticancer targets

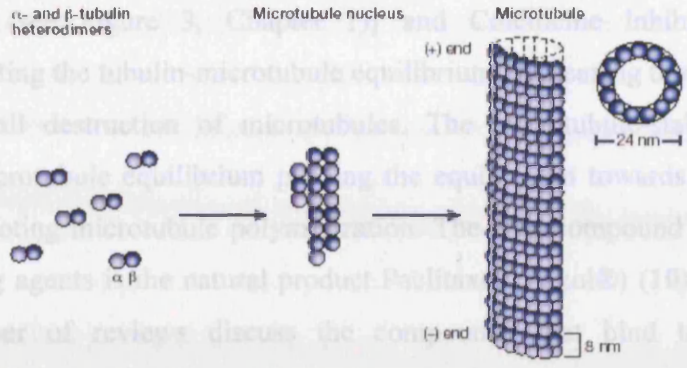
2.1 Introduction

Chemotherapy has vastly improved the survival rates of many cancers. A group of agents that has been particularly effective in the treatments of cancer are the tubulin-binding agents (also referred to as antimicrotubule agents). Tubulins are proteins that form microtubules, which are key components of the cellular cytoskeleton (structural network). Microtubules are important for diverse cellular functions including chromosome segregation during cell division (mitosis), cell structure, transport, signaling and motility. Given their primary role in mitosis, microtubules have represented an exciting target in the design of anticancer drugs. Natural and synthetic agents are known to interact with tubulin. Well known examples include Paclitaxel (Taxol®) (**10**) (see Figure 3, Chapter 1) and Vinca alkaloids. These compounds disrupt the tubulin-microtubule equilibrium, causing an overall inhibition of cell division and cell death.

2.2 Biochemistry of tubulin, microtubules and mitotic spindle

Microtubules — key components of the cytoskeleton —are long, filamentous, tube shaped protein polymers that are essential in all eukaryotic cells. They are crucial in the development and maintenance of cell shape, in the transport of vesicles, mitochondria and other components throughout cells, in cell signalling, and in cell division and mitosis. Microtubules are composed of two structurally similar protein subunits, namely α-tubulin and β-tubulin. The α and β tubulin are spherical proteins composed of approximately 440 amino acids (50 KDa). The series of events through which the α-tubulin and β-tubulin come together to form an α-β heterodimer is still not fully understood. Bound to these heterodimers are two molecules of guanosine triphosphate (GTP). One of these GTP molecules cannot be removed without denaturing the heterodimer when bound to the α-subunit (N-site). The other GTP molecule is freely exchangeable with unbound GTP when bound to the β-subunit (E-site). These heterodimers, in the presence of additional GTP and 37 °C, can combine in a head-to-tail arrangement at 80 Å intervals to form a linear protofilament.[74-77] A single microtubule is composed of thirteen protofilaments, forming a hollow structure of *ca* 240 Å diameter[78] (Figure 8).[79] The functional diversity of microtubules is achieved in several ways: through the binding of various regulatory proteins, including microtubule associated proteins (MAPs), to soluble tubulin and to the microtubule surfaces and ends; by expression of different tubulin isotypes, which have different functions; and through several post-translational modifications of tubulin.

Figure 8.



The exact purpose of these MAPs is unclear, however microtubules form faster in their presence and the MAPs also appear to protect the microtubules from conditions and agents which induce depolymerization, namely low temperature and Ca^{2+} ions. Also associated with the microtubules are Microtubule Organizing Centres (MTOCs). These MTOCs form a focus for microtubule growth, and all the microtubules initially begin to grow from one of these centres.[80] Once formed, these complex protein tubes are not static. They exist in an equilibrium with dimers constantly adding to one end of the microtubule [(+) end] and leaving at the other [(-) end]. This process of polymerization/depolymerization is referred to as dynamic instability. GTP hydrolysis at the exchangeable E-site is required to establish a flux of subunits through the polymer, adding tubulin heterodimers to the "plus" end and dissociating heterodimers from "minus" end of the microtubules, resulting in microtubule destabilization.[81,82]

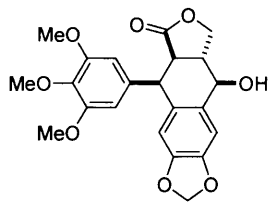
In non-dividing cells, microtubules organize the cytoplasm, position the nucleus and organelles and serve as the principal element of flagella and cilia. Cell division is a complex process undertaken by the human body. During cell division, a large dynamic array of microtubule, mitotic spindle, functions to physically segregate the duplicate chromosomes and to orient the plane of cleavage. If the microtubules in a tumor cell can be prevented from forming or decaying, the chromosomes cannot separate, the cell cannot reproduce and the tumor cannot grow.

2.3 Antimitotic agents

A large number of substances are known to bind to tubulin and/or directly to tubulin in the microtubules, inhibiting cell proliferation by blocking mitosis. Therefore, microtubule-binding drugs are often referred to as antimitotic agents. Drugs binding to tubulin can be classified in two traditional categories according to their effect on tubulin-microtubule

equilibrium. The microtubule-destabilizing agents such as the Vinca alkaloids [Vincristine (**8**) and Vinblastine (**9**) (see Figure 3, Chapter 1)] and Colchicine inhibit microtubule polymerization, disrupting the tubulin-microtubule equilibrium, decreasing the polymer mass, and causing an overall destruction of microtubules. The microtubule-stabilizing agents disrupt the tubulin-microtubule equilibrium pushing the equilibrium towards the assembled microtubule and promoting microtubule polymerization. The lead compound of the class of microtubule-stabilizing agents is the natural product Paclitaxel (Taxol®) (**10**) (see Figure 3, Chapter 1). A number of reviews discuss the compounds that bind to tubulin and microtubules.[74,80,83,84] Here, a discussion of those compounds most relevant to this thesis is provided. Inhibition of Tubulin Polymerization will be abbreviated as ITP.

Microtubule-binding drugs can also be grouped according to their binding site on tubulin. For instance, Paclitaxel is known to stabilize microtubules[85] and the functional "Paclitaxel binding site" has been located on the β-tubulin.[86] Drugs targeting the paclitaxel-binding site are known to act as microtubule-stabilizing agents.[74] The vinca domain and the colchicine sites are the other well established drug binding sites, located on the β-tubulin.[74] Agents such as Vincristine (**8**), Vinblastine (**9**) clearly defined the vinca binding site. Molecules such as Podophyllotoxin (**32**), Combretastain A4 (**15**) (Figure 9) are known to occupy the Colchicine binding site and block microtubule formation.[74] A number of these agents are effective anticancer drugs.



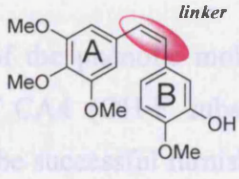
Podophyllotoxin (**32**)

The clinical success of several vinca alkaloids and taxanes for the treatment of human cancers has validated microtubules as a target and has encouraged the search for compounds sharing a similar mode of action.

Besides their ability to inhibit tumour cell proliferation, some microtubule-targeting agents display toxicity towards tumor vasculature, inducing occlusion of preexisting tumour blood vessels, producing tumour cell death from ischemia and necrosis.[28] Molecules such as the vinca domain agents such as Vincristine (**8**), and Vinblastine (**9**), the taxane agents[87] and Combretastain A4 (15) have been shown to destroy neovasculature, but except for CA4, this effect is observed close to the maximum tolerated dose. Moreover, the antivasculature effect is most pronounced for agents binding at the colchicine-binding site. The agents CPA4 (**16**) [a water soluble prodrug of CA4 (**15**)], AVE8062 (**19**) and ZD6126 (**20**), are all in Phase I/II clinical trials (see Figure 5, Chapter 1). The therapeutic effect of these agents such as CA4P (**16**) appears to derive from their vasculature targeting properties and not antimitotic properties. This vasculature targeting

approach has attracted several[88] several research groups worldwide to undertake studies aimed at evaluating natural and synthetic products structurally related to CA4. A large number of CA4 analogues have been prepared and evaluated for their cytotoxicity, antitubulin and anticancer properties. From a synthetic and medicinal chemistry perspective, the search and development of CA4 analogues has been encouraged by the simplicity of its structures and the enormous chemical diversity that can be introduced in a such simple template. The scaffold of CA4 presents three points of diversity amenable of modification: the A ring, the linker, and the B ring (Figure 9).

Figure 9. Points of diversity of the CA4 (**15**).



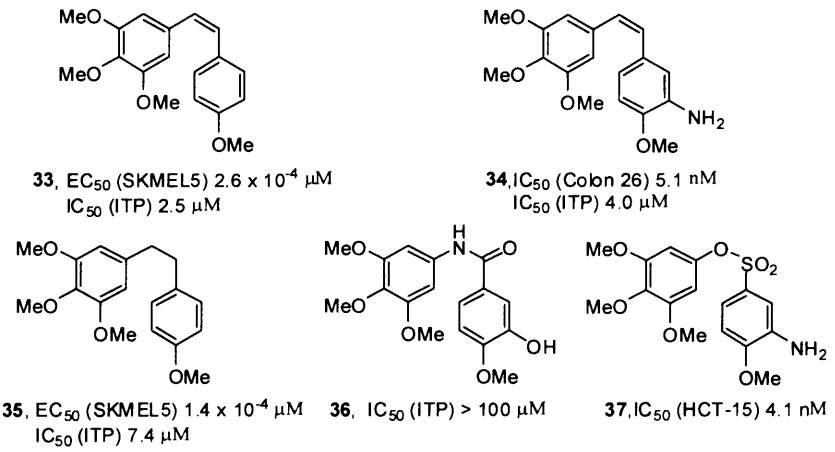
Combretastatin A4 (**15**)

IC_{50} (ITP) 2.0 μM
IC_{50} (Colon 26) 18.0 nM
EC_{50} (SKMEL5) 1.4 x 10^{-4} μM
IC_{50} (HCT-15) 1.7 nM
IC_{50} (B16) 1.0 nM
IC_{50} (K562) 4.3 nM

The trimethoxy substituted phenyl ring present in CA4 (**15**) is an important feature partly responsible for the cytotoxicity and the strong binding to tubulin. This chemical motif is also a recurrent feature of other antitubulin agents (e.g. Podophyllotoxin, Colchicine). Replacement of the *meta* methoxy group with a hydroxyl,[89] as well as the removal of the *meta* or *para* methoxy group[90] resulted in a significant drop in potency. Loss of activity was also observed when an unsubstituted phenyl ring was present.[90] Replacement of the methoxy groups with bulkier groups such as ethoxy was not tolerated.[91] Attempts to replace the trimethoxyphenyl ring with more lipophilic groups such as trimethylbenzene or naphthalene resulted in significant decrease of cytoxicity.[91,92]

A detailed SAR has been conducted around the B ring.[84] The requirement of the *para* methoxy group for cytotoxicity has been established by Cushmann and coworkers.[90,93] Loss in activity was also observed for the *para*-ethoxy and *para*-propoxy derivatives.[90] Changing the position of the methoxy group from *para* to *meta* position resulted in a dramatic loss of potency.[93] It has been suggested that the oxygen of the *para*-methoxy group act as a hydrogen bond acceptor. Therefore its replacement with sulfur was not tolerated.[93] The comparable biological activity of compounds **15** and **33** (Figure 10) reveals that the hydroxyl group is not essential.[90,93]

Figure 10. Analogues of CA4 (**15**).



Several replacement of the phenolic moiety have been attempted in order to improve the metabolic stability of CA4 (OH-F substitution) retaining the potency.[94] The OH→NH_2 substitution proved to be successful furnishing compound **34** displaying an improved potency compared to CA4 (**15**).[95] Moreover, replacement of the hydroxyl group with a boronic acid[96] or azide[97] appears to be tolerated. Finally, the replacement of the B ring with naphthalene[98] and quinazoline[99] system proved to be successful.

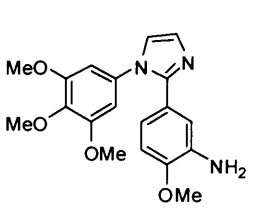
Modification of the olefinic linker has received major attention. It is believed that the spatial relationship between the two aromatic rings is an important feature that determines the ability to bind to tubulin, maximizing the interaction with the target. The *Z* configuration of the double bond appears to be optimal for good activity.[93,100,101]

Synthetic efforts have been directed towards the design of linking groups capable of positioning the two aryl rings in a way that results in good activity. Reduction of the double bonds afforded compound **35**[90] (Figure 10) displaying modest antitubulin properties. The olefinic bridge appeared to be the most active linker.

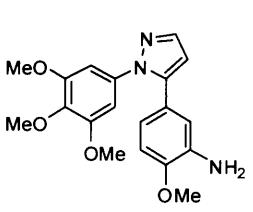
Replacement of olefinic bond with a single oxygen linker resulted in loss of cytotoxicity and antitubulin activity due to the short distance between the rings.[90,102,103] The effect of several other substitutions has been object of detailed biological investigation. For instance, the CH→NH or CH→O substitutions have been attempted affording compounds with some biological activity, although significantly decreased compared to CA4.[90,103] The amide **36**[104] (in which the amide acts as a bioisosteric replacement of the alkene) displayed a dramatic loss in cytotoxicity and antitubulin activity, while the sulfonate group (compound **37**) appeared to be well tolerated (Figure 31).

Several analogues of CA4 where the olefinic ring is replaced by a ring were also synthesized. These modifications provide *cis*-locked analogues of CA4, preventing combretastatin *cis* → *trans* isomerization, but still maintaining the free rotation of the two aromatic rings. Furthermore, the replacement of the double bond linker with a ring may lead to potent CA4 analogues possessing an optimum pharmacological profile. From a medicinal chemistry perspective, five-membered heterocycles compounds represented an attractive target. Among the synthesized compounds, (e.g. imidazole,[105] 1,3-oxazole,[105] pyrazole,[105] triazole,[106] furazan,[107] 1(5H)-furanone,[108] diaryloxazolones,[109] 2-cyclopenten-1-one,[110] etc), most of them retain the cytotoxicity and antitubulin activity (Figure 11).

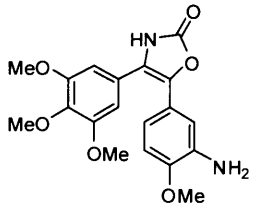
Figure 11. Analogos of *cis*-restricted CA4 (**15**).



38,IC_{50} (HCT-15) 69 nM
IC_{50} (ITP) 7.7 μM



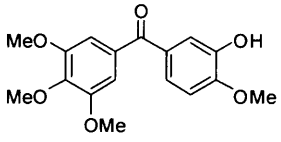
39,IC_{50} (HCT-15) 28 nM



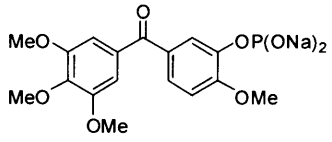
40,IC_{50} (B16) 2.4 nM

Series of benzophenones and related ketones were evaluated for cytotoxicity and their ability to inhibit tubulin polymerization. Lead compounds of this series are phenstatin (**41**), and phenstatin phosphate (**42**) discovered and described by Pettit and coworkers[111] (Figure 12). Independent studies led to the identification of numerous benzophenones, structurally related to phenstatin (**40**) (e.g. compounds **43-46**)[112,113] exhibiting potent cytotoxicity and modest antitubulin properties.

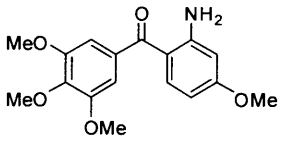
Figure 12. Structure of benzophenones and ketones structurally related to CA4 (**15**).



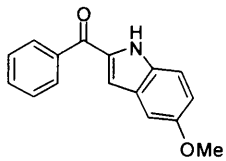
41
, IC_{50} (OVCAR-3) 2 nM
IC_{50} (ITP) 1.0 μM



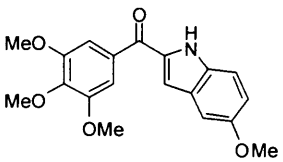
42, IC_{50} (OVCAR-3) 2 nM
IC_{50} (ITP) 21 μM



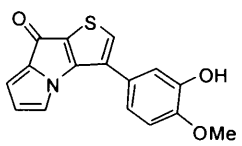
43, IC_{50} (KB) 26 nM
IC_{50} (ITP) 82 μM



44, IC_{50} (Hela/KB) 68 nM
IC_{50} (ITP) 0.53 μM



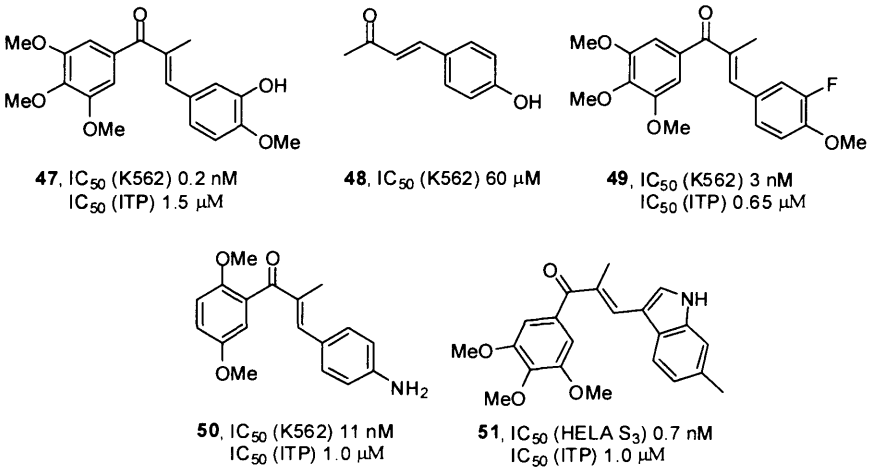
45, IC_{50} (Hela/KB) 29 nM
IC_{50} (ITP) 0.99 μM



46, IC_{50} (Hela/KB) 15nM

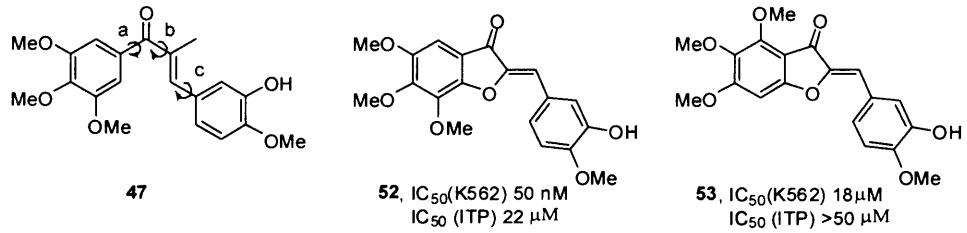
Within our own research group, significant cytotoxicity and antitubulin properties were observed for chalcones (Figure 13), an additional series of compounds bearing a α,β unsaturated linker where the two phenyl rings are separated by three atoms. The α-methyl-chalcone **47**[114,115] (Figure 13) emerged as a potent from a detailed SAR studies,[116-118] conducted to further develop the initial lead **48**, isolated from the Chinese herb *Scutellaria barbata*. The chalcone **47** displayed potent ability to inhibit tubulin assembly by binding the Colchicine-binding site of tubulin.[114]

Figure 13. Structure of chalcones **47** and its analogs.



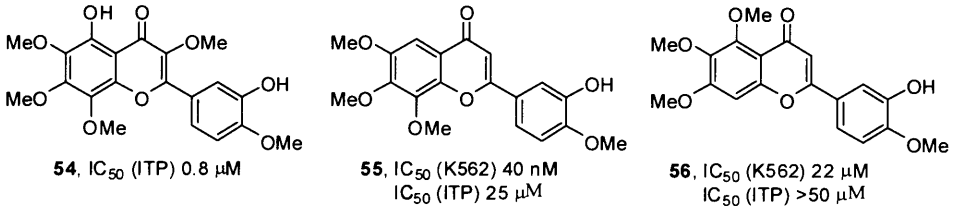
The X-ray crystal structure of **47** revealed that the carbon-oxygen and carbon-carbon double bonds are positioned *trans* relative to the C1-C2 single bond. Preliminary modeling and crystallographic studies led us to postulate that molecules adopting the *s-trans* conformation bind strongly to tubulin. The presence of the carbonyl group also appeared to be essential for potency, presumably due to a hydrogen bond with Leu255 NH.[115] Related analogues of chalcone **47** have been synthesized to date. The fluorine analogue **49**[94,115,119] was prepared to overcome the poor bioavailability of **47**, and avoid unwanted metabolic degradation. It showed less cytotoxicity than **47**, but is still potent. Other examples include the amino derivative **50**[117,120-123] and indoles **51**[124] found to be potent cytotoxic and good antitubulin agents. We also investigated the anti-tubulin activity of numerous conformationally-restricted analogs of **47** (Figure 14).

Figure 14. Aurones, conformationally-restricted analogs of **47**.



A series of aurones were synthesized as conformationally-restricted analogues of **47** and evaluated for cytotoxicity their antitubulin properties (Figure 14).[125] The major goal of this modification was to get an insight into the importance of the aryl ring orientation about the rotatable bond a and c in influencing the cytotoxicity and anti-tubulin properties (Figure 14). SAR studies revealed that the 5,6,7-trimethoxyphenyl moiety of aurone **52** is optimum for relevant cytotoxicity and antitubulin activity. Loss of potency was observed for the 4,5,6-trimethoxy isomer **53**. However, the aurone derivatives were found to be significantly less active than chalcone **47**, indicating the importance of the rotational freedom around bond a.
Several natural[126] and synthetic flavones were evaluated for their tubulin-binding properties and cytotoxicity. The natural product **54** emerged as one of the most active compounds (Figure 15).

Figure 15. Flavones, conformationally-restricted analogs of **47**.



Synthetic derivative **55**[125] exhibited an enhanced potency compared to **54** (Figure 15). Moreover, the presence of the 6,7,8-trimethoxy A ring appeared to be optimal for good activity as shown by the reduced potency of the corresponding 5,6,7-trimethoxy derivative **56** (Figure 15). Many other analogues structurally related to flavones were synthesized and evaluated as potential tubulin binding agents. These include quinolones,[127-130] quinazolines,[131-133] and naphthydyridones.[134,135]

2.4 Conclusion

In light of the vascular-damaging properties of the combretastatins, the potential of tubulin and microtubules in cancer therapy has been reevaluated. The investigation of new and more potent compounds related to Combretastatin A4 (**15**) with improved pharmacological properties and agents binding to colchicine binding site has gained great interest yielding a large number of promising compounds. Continued investigation of how the microtubule-targeting agents exert their antiangiogenic activity is likely to lead to significant clinical advances in cancer treatment.

Chapter 3

3.0 STAT3: an attractive target for anticancer tharapy

3.1 Introduction

Signal transducers and activator of transcription (STAT) proteins comprise a family of transcription factors that consist of seven members: STAT 1, 2, 3, 4, 5A, 5B, and 6.[136] They are latent in the cytoplasm and participate in normal cellular events, such as differentiation, proliferation, cells survival, apoptosis and angiogenesis in response to cytokines, growth factors and hormones signaling.[137-140]

STAT3 is the transcription factor whose critical role in oncogenesis has been amply studied and described. STAT3 is activated by tyrosine phosphorylation (Tyr705). In contrast to normal signaling, in which STAT3 phosphorylation is transient and a tightly regulated process, aberrant constitutive activation of STAT3 has been detected in over a dozen of types of human cancers.[141,142] Several pieces of evidence have been provided that persistently-activated STAT3 signaling contributes to disrupt normal physiological control and leads to oncogenic transformation. Studies have demonstrated that inhibition of STAT3 signaling in tumour cells results in apoptosis, suppression of angiogenesis and stimulation of immune response.[143-145] It has been observed a dependence of tumour cells on persistent STAT3 activation and an increased sensitivity to STAT3 inhibition than normal cells. This has important implications for cancer therapy, providing the potential selectivity for tumour cell killing.[144] Keeping all these findings in mind STAT3 is believed to be a potential target for cancer therapy.[146]

3.2 Background

STAT proteins are activated by a ligand receptor binding at the cell surface.[147] The class of ligands comprises a variety of factors such as cytokines, growth factors, and hormones. The receptors are transmembrane proteins. When the receptor is bound to its ligand, its dimerization occurs. The dimerized receptors, unlike those with intrinsic tyrosine kinase activity, induce STAT tyrosine phosphorylation by recruiting the receptor-associated tyrosine kinase such as Janus kinases (JAKs)[148] or Src kinases family.[149,148] Other possible tyrosine kinases that can phosphorylate STATs are peptide growth factors receptors such as EGFRs (epidermal growth factors receptors). Once it has become activated, the JAK kinase causes the phosphorylation of a specific tyrosine residue within the cytoplasmatic tail of the receptor. This provides docking sites for the recruitment of STATs which are activated by tyrosine phosphorylation. Phosphorylated proteins dimerize by reciprocal interaction of their SH2

domains and phosphotyrosine residues.[150,151] Dimerized STATs translocate to the nucleus,[152] bind to a specific DNA sequence, and regulate gene expression[153,144] (Figure 16).

Figure 16. Normal and oncogenic STAT3 signaling pathway.

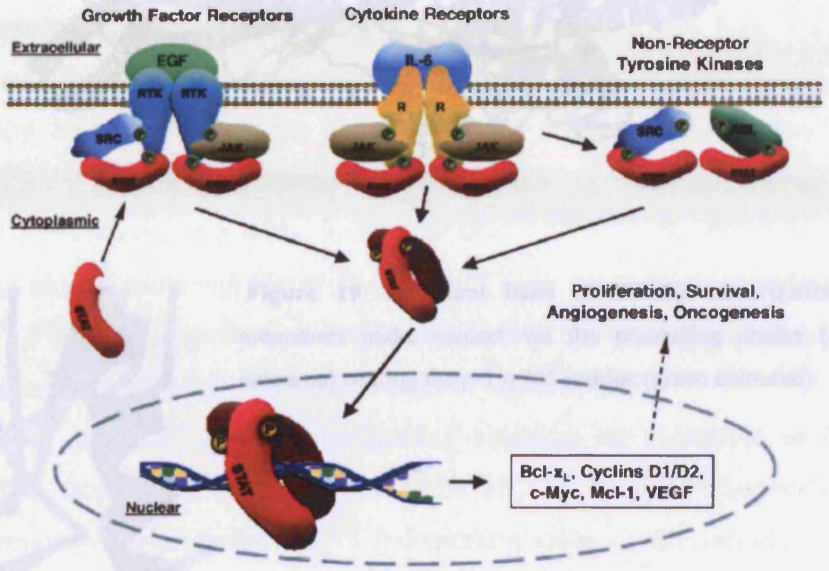


Figure 17. X-ray of STAT3 dimer disclosed in 1998.

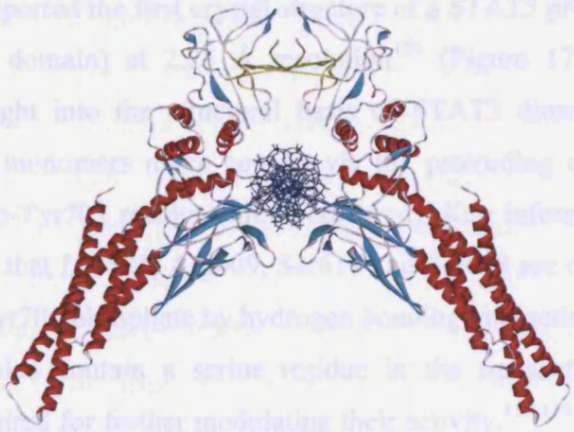
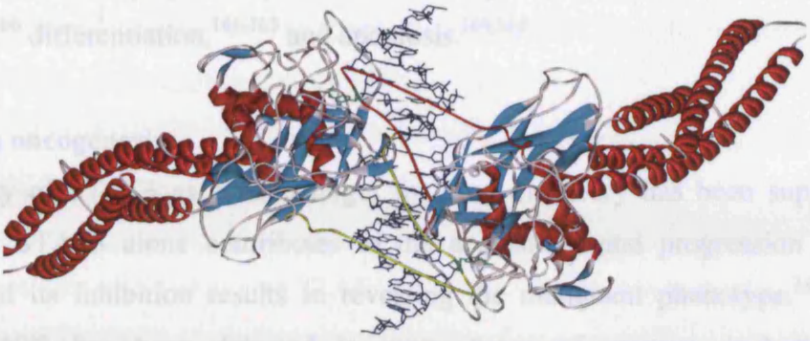


Figure 18. SH2 domain dimerization interface of STAT3b protein.



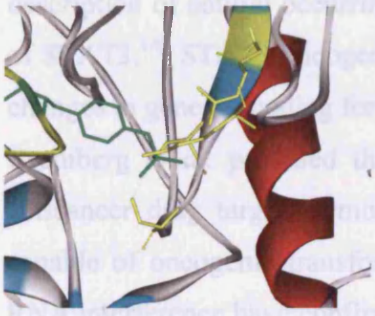


Figure 19. Structural basis of STAT3 dimerization. The STAT3 monomers make contact via the protruding chains (red and yellow coloured) bearing the p-Tyr705 residue (green coloured).

Becker *et al.* in 1998 reported the first crystal structure of a STAT3 protein bound to its DNA recognition site (SH2 domain) at 2.25 Å resolution[154] (Figure 17 and 18). The crystal structure provides insight into the structural basis of STAT3 dimerization. As shown in Figure B, the STAT3 monomers make contact via the protruding chains (red and yellow coloured) bearing the p-Tyr705 residue (green coloured). Key information of the p-Tyr705 binding cavity indicate that Lys591, Arg609, Ser611 and Ser613 are directed involved in the stabilization of the p-Tyr705 phosphate by hydrogen bonding interactions (Figure 19).

STAT1 and STAT3 also contain a serine residue in the transactivation domain whose phosphorylation is required for further modulating their activity.[155-159] In normal cells STATs activation is a transient process and within hours the activating signals decay and the STATs are exported back to the cytoplasm. Although some aspects of the negative regulation have been elucidated, the overall mechanism by which the activating signals decay are not fully understood.[147] Several classes of negative regulators recently discovered,[159,155] include SH2-containing protein tyrosine phosphates (SHP), the suppressor of cytokines signaling (SOCS) proteins, and the proteins inhibitors of activated STATs (PIAS). STAT proteins can be divided into groups according to their physiological functions. Specifically, STAT3[141] has

been demonstrated to play a key role in the regulation of different events including proliferation,[160] differentiation,[161-163] and apoptosis.[164,165]

3.3 STAT3 in oncogenesis

The credibility of STAT3 as a valid target for drug discovery has been supported by the evidence that STAT3 alone contributes to the acquisition and progression of malignant phenotype and its inhibition results in reversing the malignant phenotype.[142] Persistently activated STAT3 has been detected in many types of cancers, including leukemia, lymphomas, carcinomas, and other solid tumours.[143,144,166,167] So far there has been no description of natural occurring mutation of STAT3 gene resulting in constitutive expression of STAT3.[142] STAT3 oncogenic signaling is the result of indirect effect of mutation–induced changes in genes encoding for STAT3 activators or repressors.

Bromberg *et al.* provided the most compelling evidence for validation of STAT3 as an anticancer drug target, demonstrating that while STAT3 is made constitutively active, is capable of oncogenic transformations.[168] Independent studies with antisense, gene therapy, RNA interference have confirmed that STAT3 signaling inhibition reduce tumour growth and induce apoptosis in cell lines and mouse models.[169-171]

STAT3[141,144] plays its role in tumourgenesis through up-regulation of genes encoding for apoptosis inhibitors (e.g Bcl-x_L), cell-cycle regulators (e.g. cyclin D_1), and inducers of angiogenesis (e.g vascular endothelial growth factors-VEGF) (Figure 16). The role of STAT3 in regulating p53 (the so called "tumour suppressor") expression and function has been investigated. A reduced p53 activity has been detected in many types of cancer[172] and experimental evidences have identified in STAT3 a mediator of p53 suppression.[173]

On the basis of these observations the inhibition of STAT3 signaling pathways is expected to result in downregulation of the expression of several oncoproteins and reactivation of p53 expression and function in diverse human cancers.

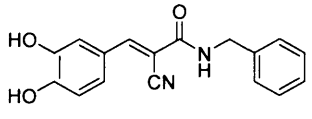
3.4 Targeting STAT3: strategies for drug discovery

Expanding understanding of the mechanism of constitutive STAT3 activation has provided a rational basis to target constitutive STAT3 signaling pathways. As shown in Figure 16, in the STAT3 pathways there are potential sites for intervention to induce disruption of STAT3 function. The strategies can be generally divided into direct or indirect. The indirect approaches focus on inhibiting the STAT3 pathways by targeting the upstream key activators or potentiating the negative regulators.

Direct targeting of STAT3 can be achieved by inhibiting its expression or disrupting different aspects of its function such as recruitment, phosphorylation, dimer formation, nuclear translocation, DNA binding, gene transcription.

3.4.1 Indirect STAT3 targeting strategies

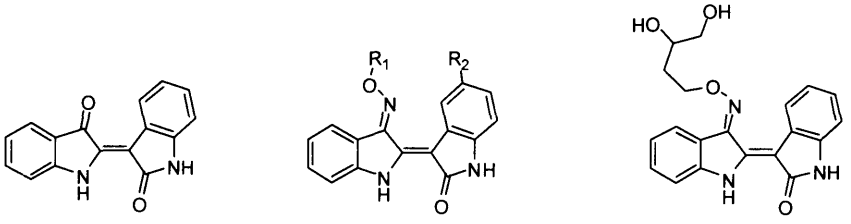
Given the success of tyrosine kinases selective inhibitors as anticancer therapy, inhibition of tyrosine kinases activity upstream of STAT3 pathways has represented an appealing strategy to prevent aberrant STAT3 activation and related malignant transformation (Figure 16). Src and JAK families and EGFRs represent potential targets for drug discovery.



Tyrphostins AG490 (**57**)

For instance, Tyrphostins AG490 (**57**) is a specific and potent JAK-2 protein tyrosine kinase inhibitor. Several studies have shown that AG490 reduces STAT3 DNA-binding activity[174] and selectively inhibits leukemia cells growth *in vivo* and *in vitro* inducing apoptosis.[175-177] Nam *et al.* have recently reported that indirubin derivatives such as E564 (**59**), E728 (**60**) and E804 (**61**) block constitutive STAT3 signaling in human breast and prostate cancer cells (Figure 20).[178] In addition E804 has been identified as c-Src kinase inhibitor (IC_{50} = 0.43 μM) *in vitro*. Reduction of phosphotyrosyl c-Src levels have been detected in cultured cells after E804 treatment. Tyrosyl phosphorylation of STAT3 and constitutive STAT3/DNA binding activity were suppressed resulting in apoptosis. E804 was initially tested as a racemate. However, the two enantiomers, prepared and separately screened, showed a similar inhibition of phosphorylation of Src and STAT3, suggesting that the configuration of the chiral centre does not effect the binding to the target and the activity. Nam suggested the hypothesis that E804 as an ATP-mimic may bind to the ATP binding site of the Src tyrosine kinase.

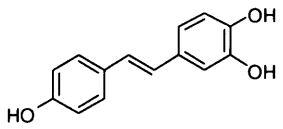
Figure 20. Structures of indirubin and indirubin derivatives.



Indirubin (**58**)

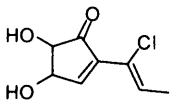
E564 (**59**) $R_1 = (CH_2)_2O(CH_2)_3OH$, $R_2 = H$
E728 (**60**) $R_1 = H$, $R_2 = OCH_3$

E804 (**61**)



Resveratrol (**62**)

Antitumour properties of the well known natural compound Resveratrol (**62**) associated with its ability to inhibit the Src/STAT3 signaling pathway[179] have been recently reported. Resveratrol inhibited Src tyrosine kinase activity and blocked aberrant STAT3 activation in malignant cells inducing apoptosis. By contrast, cells treated with resveratrol, but lacking aberrant Stat3 activity, showed reversible growth arrest.



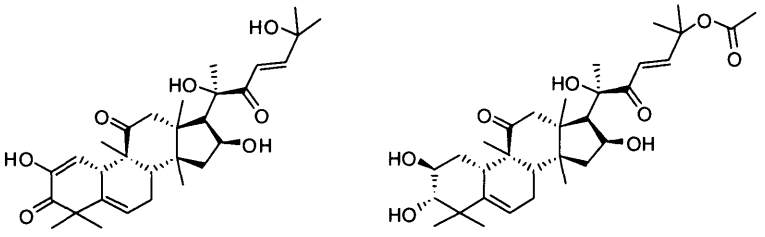
CPDHC (**63**)

Targeting of serine kinases has been suggested as an additional therapeutic strategy[143] based on the evidence that elevated serine kinase activity is associated with oncogenesis[180] and increased levels of serine phosphorylation of STAT1 and STAT3 have been detected in chronic lymphocytic leukemia.[181] Turkson et al. have demonstrated that pharmacological inhibition of serine phophorylation results in blockage of transformation induced by Src oncoprotein[182] providing evidences for the potential of serine kinases as targets for cancer therapy. So far there are no examples of systematic investigations of specific serine kinases inhibitors that block STAT3 signaling pathway. However, in the screening of a fungal extract Weidler and coworkers identified CPDHC (**63**), a cyclopentanone derivative, as an inhibitor of the IL-6 dependent JAK/STAT signaling cascade.[183] Studies on the mode of action revealed that CPDHC inhibits the JAK/STAT pathways involving the direct inhibition of the Janus kinase as well as an unidentified serine kinase responsible for the phosphorylation of serine 727 of STAT3.

Sebti and coworkers identified JSI-124 (Cucurbitacin I) (**64**), as a selective JAK-STAT3 inhibitor (Figure 21). The discovery is based on the use of high throughput screening of the NCI Diversity Set of 1,992 compounds.[184] JSI-124 is a natural product isolated from different plants such as Cucurbitaceae and Cruciferae, used as a folk remedy for centuries in China and India. It has been demonstrated to suppress phosphotyrosine levels of STAT3 and JAK-2 in many human cancer cell lines, and, as a consequence, reduce STAT3 DNA-binding activity and STAT3-mediated gene transcription resulting in the inhibition of tumour growth. The mode of action of JSI-124 has not been elucidated. Sebti *et al.* suggest that JSI-124 could promote the protein phosphatase activity of SHPs or could activate suppressors of cytokine signaling, STAT-induced STAT inhibitors, JAK-binding protein, and STAT3-interacting proteins. Further studies led to the discovery of Cucurbitacin Q (**65**), which inhibits the activation of STAT3 and induce apoptosis without inhibiting JAK2[185] in A549 cells (a human non-small-cell lung carcinoma line) (Figure 21). The discoveries were used as probes to

prove that suppression of STAT3 activation, not JAK2 function, is more deleterious for tumour survival. This finding validated further STAT3 as a drug target to fight cancer.

Figure 21. Structures of Cucurbitacin I (**64**) and Cucurbitacin Q (**65**).



JSI-124
Cucurbitacin I (**64**)

Cucurbitacin Q (**65**)

Overexpression of the EGFR (epidermal growth factor receptor) family has been detected in many types of cancer.[186,187,188] The literature reports several studies validating the principle that inhibition of tyrosine kinase activity of EGFR is effective in downregulating STAT signaling and tumour growth.[189] Employing specific tyrosine kinases inhibitors in targeting EGFR may constitute a viable approach for abrogation of STAT3 activation. Disruption of the phosphorylation of specific EGFR tyrosine residue by EGFR-specific peptide aptamers have been proved to result in inhibition of EGFR-mediated STAT3 activation.[190] An alternative strategy employs receptor or ligand antagonists, a class of molecules that lack the intrinsic activating properties of the physiological ligand and possess higher affinity for receptor. It has been demonstrated that the aberrant IL-6 cytokine signaling pathways is responsible for constitutive activation of STAT3 signaling and consequently, for the malignant progression of multiple myeloma.[174] The "superantagonist", Sant7, an IL-6 variant, has been found to downregulate the constitutive STAT3 activation in myeloma cells[174] and inhibit tumour growth[191,192] by blocking the IL-6 receptor activation.[193] Active research is currently ongoing in order to provide the preclinical rationale for clinical trials of Sant7.[194] Finally, antisense oligodeoxynucletide (ODN) strategy to degrade selectively STAT3 mRNA has been demonstrated to inhibit tumour growth in different cell lines.[195,196]

3.4.2 Direct STAT3 targeting strategies

Dimerization is the key step in the STAT3 activation. After dimerization STAT3 translocates into the nucleus and binds to a specific DNA sequence inducing gene transcription. These three events, dimerization, translocation and DNA-binding, represent very appealing targets for inhibition of oncogenic STAT3 signaling pathways. Significant efforts have been done to

identity phosphopeptides, peptidomimetics and small molecule inhibitors of STAT3 and develop antisense strategies.

To interfere with STAT3 dimerization, ideal compounds should possess certain properties, including a strong affinity for STAT3 monomer that favors the generation of a heterocomplex of STAT3-compound over the STAT3-STAT3 dimer.[167] The association of the compound with pre-existing dimers of STAT3 might: a) facilitate the dissociation of STAT3 dimers and the preferential formation of a heterodimeric complex involving STAT3 and compound; b) generate a heterotrimeric complex that would interfere with STAT3-dimer ability to bind to DNA.[167]

A novel approach for disrupting the DNA binding of STAT3 molecules relies on the use of G-quartet-oligodeoxynucleotides (GQ-ODNs)[197] as STAT3 inhibitors. GQ-ODNs have been shown to interact with the SH2 domains of the STAT3 dimers *in vitro*[198] *and in vivo.*[199,200]

Disrupters of STAT3 dimerization could also be small peptides, small-peptide mimetics or small molecules that are specific for the SH2 sequence of STAT3. The reported crystal structure of STAT3 dimers bound to DNA should provide insight for the design of small molecule inhibitors targeted at the SH2 domain (Figure 17).

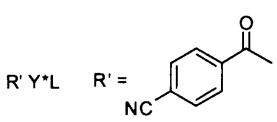
Identification of phosphopeptides that bind to the SH2 domain of STAT3 is an approach to target STAT3 pursued by several groups. Turkson *et al.* published the first example of development of phosphotyrosyl peptide STAT3 inhibitor targeted to the SH2 domain[201]. They investigated and demonstrated the ability of the phosphopeptide PY*LKTK (where Y* represents phosphotyrosine) — derived from the native STAT3 amino acid sequence in the vicinity of Tyr^{705} in the SH2-binding domain — to disrupt STAT3 activity *in vitro*. *In vitro*, PY*LKTK phosphopeptide inhibits STAT3/DNA binding activity with IC_{50} values of 235 μM, binding to the SH2 domain of STAT3, disrupting the Tyr(P)-SH2 interactions that stabilize active STAT3:STAT3 dimers and forming inactive STAT3:PY*LKTK heterocomplex. Furthermore the presence of Tyr(P) in the peptide sequence was critical for reduction of STAT3/DNA binding activity. Phosphopeptides PYLKTK and PFLKTK had no effect on STAT3/DNA binding activity at significant concentrations. Structure-activity studies of PY*LKTK led to the identification of tripeptide derivatives PY*L and AY*L as inhibitors of STAT3 activation and biological function.

In a similar fashion, Ren *et al.* tested a panel of peptides known to bind to SH2 domains of STAT3. The goal of this study was the discovery of a lead peptide for peptidomimetic drug development. A series of tyrosyne-phosphorylated hexapeptides were evaluated for their ability to impede STAT3/DNA binding by disrupting receptor recruitment and/or

STAT3:STAT3 dimer formation.[202] The most active compound showing great activity (IC_{50} 0.15 μM) was based on Gp130 amino acid sequence Y*LPQTV. The suggested mode of action is the destabilization of STAT3:STAT3 dimer by direct Tyr^{705}-SH2 interaction.

The development of phosphopeptides as clinically useful drugs is limited by their poor metabolic stability (or instability to peptidases and phosphatases), low bioavailability and low cellular permeability. Peptide medicinal chemistry has been actively engaged in developing strategies to produced modified peptides with reduced peptide character and "drug-like" properties reproducing or enhancing the activities of the original peptide. Under this principle, Turkson and coworker pursued a semi-rational peptidomimetic approach aiming at generating a series of new inhibitors with a reduced peptide character.[203]

The tripeptide lead compounds, PY*L (IC_{50} 182 μM, σ 15, *in vitro* assay) and AY*L (IC_{50} 217 μM, σ 55, *in vitro* assay) previously discovered, were modified by substituting the proline and alanine residue by aromatic groups and replacing the peptide bond that is the NH_2-terminal to the phosphotyrosine (P). The resulting series of pepitodomimetics (of generic structural formula R'Y*L) were evaluated for their ability to disrupt STAT3/DNA binding activity *in vitro*.



ISS 610 (**66**), IC_{50} = 42 μM

The most potent compounds obtained showed IC_{50} values that range between 75 and 38 μM. ISS610 (**66**) was chosen as representing compound for cell studies to investigate its ability to reproduce the biological activity of the phosphopeptide. It was found to inhibit constitutive STAT3 activity in different types of cells inducing cell growth inhibition and apoptosis. What is promising, however, is the fact that peptidomimetic approach can be undertaken to design relatively small peptidomimetics and, ultimately, small non-peptide inhibitors of STAT3, that can be recognized by sites that bind to larger peptides.

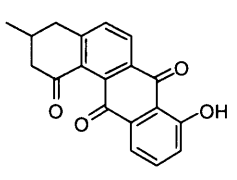
Targeting protein-protein interaction has represented a hard and challenging goal to achieve for drug discovery. The identification of "drug-like" small molecule inhibitors of protein-protein interactions and their function has proved difficult. However, in view of the increasing number of publications reporting small-molecule inhibitors of protein-protein interactions,[61,73] significant efforts have been made in pursuing the small molecule-based approach to target directly STAT3.

Computer-based screening strategy represents a valuable approach to identify small-molecules disrupting protein-protein interaction as a primary mode of action. Knowledge of the three-dimensional structure of a target, obtained using X-ray crystallography provides a notable improvement for the rational design of specific inhibitor molecules that target

functionally important parts of the structure. Moreover, the Protein Data Bank archive (PDB) of macromolecular structural data is freely available in the public domain providing a variety of tools for studying the structures of biological macromolecules and their relationships to sequence, function, and disease. Databases of virtual libraries of small molecules organic compounds (for which the three-dimensional structural models have been defined or could be generated from the 2D chemical structures by using the appropriate computational program) are also freely provide by the National Cancer Institute (NCI), or are commercially available from other chemical catalogs (i.e. Merck Index, Sigma-Aldrich). A fruitful application of the increasing number of structural data is the so-called *in silico* screening of virtual libraries of compounds against a known structure of a protein target. The structure-based design can be used as a productive approach for generating initial leads with the advantage that the virtual libraries can contain a very large number of compounds characterized by a high chemical diversity. Furthermore, drug-like[204,70] and ADME (absorption, distribution, metabolism, excretion) features can be easily incorporated in the early in silico-screening stage of the discovery cycle. Once the region targeted for docking is defined, molecular docking program can be used to predict the binding model and estimate the binding affinity of the compounds. The best-scored compounds can be evaluated for their activity *in vitro* and *in vivo*.

The first report of a low-molecular-weight compound inhibitor of STAT3 function discovered through virtual database screening[205] has been recently published. In this study a virtual library of 429,000 compounds was screened by computational screening to identify potential small molecule inhibitors of STAT3. The crystal structure STAT3β solved in 2.25-Å resolution (Figure 17, 18, and 19)[154] (pdb ID code 1BG1) was used.

Of the 100 best-scored compounds selected as candidates for biological testing, STA-21 (**67**) showed a significant inhibition of STAT3 dimerization, DNA binding, nucleus translocation and STAT3-regulated antiapoptotic factors such as Bcl-x_L and cyclin D1 in breast carcinoma cells.



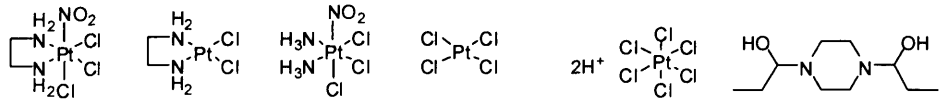
STA-21 (**67**)

The phosphorylation of STAT3 upstream regulators JAK2, Src, and EGF receptors were not affected by STA-21. STA-21 reduces the survival of breast carcinoma cells with constitutive STAT3 signaling but has a minimal effect on the cells in which constitutive STAT3 signaling is absent.

Novel platinum (IV)-containing compounds have been also evaluated for their ability to disrupt STAT3 activity.[206] The platinum derivatives CPA-1 (**68**), CPA-3 (**69**), CPA-7 (**70**) and and platinum(IV) complex **71** exhibit inhibitory effect on *in vitro* STAT3-DNA binding

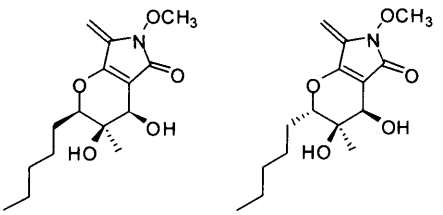
activity (IC_{50} = 5.0 μM, IC_{50} = 1.5 μM, and IC_{50} = 5.8 μM, IC_{50} = not reported, respectively) suggesting a direct interaction of the platinum compounds with the protein. CPA-1 (**68**), CPA-7 (**70**), and the platinum(IV) complex **71** have been found to inhibit cell growth and induce apoptosis in malignant cells characterized by persistently active STAT3 (Figure 7).[206]

Figure 22. Structures of the platinum derivatives CPA-1 (**68**), CPA-3 (**69**), CPA-7 (**70**), and platinum(IV) complex **71**, and IS3 295 (**72**).



CPA-1 (**68**) CPA-3 (**69**) CPA-7 (**70**) Platinum (IV) tetrachloride (**71**) IS3 295 (**72**)

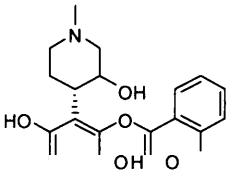
Cells that do not contain constitutive active STAT3 were marginally affected or were not affected by these compounds. CPA-7 (**70**) was the most potent compound *in vitro*, in the whole cells and induced inhibition of STAT3 and tumour regression in the animal models. In contrast, absence of inhibitory effect against STAT3 was observed for CPA-3 (**69**), which is a platinum (II) complex in vivo, indicating the possibility of different modes of action occurring in the cells for platinum (II) compounds. The influence of the oxidation state of platinum on the biological activity of CPA-1 (**68**) and CPA-7 (**70**) needs to be addressed and further investigated. Further studies led to the identification of a new platinum(IV) compound, IS3 295 (**72**), as a potent STAT3 inhibitor (Figure 22).[207] *In vitro* DNA-binding assay IS3 295 inhibits STAT3 activated dimer with an IC_{50} of 1.4 μM. Further experiments have shown that IS3 295 interacts with the inactive STAT3 monomer but lacks of inhibitory effect when the protein is pre-bound to DNA. In transformed cells the observed biological effect of IS3 295 is the repression of the expression, STAT3-mediated genes such as Bcl-x_L, cyclin D, and VEGFs, blockade of cell cycle progression and proliferation and induction of apoptosis. These findings suggest that the antitumour properties of the platinum(IV) compounds **71** may derive from their anti-STAT3 activity. However the mode of action has not been elucidated and interactions with other signaling proteins might contribute to the overall effect and cannot be excluded.



Phaeosphaeride A (**73**) Phaeosphaeride B (**74**)

In the effort to discover novel small molecules inhibitors of STAT3, Maloney *et al.* evaluated a library of 10,000 compounds and natural product extracts and organic extracts of fungal culture for their ability to disrupt STAT3/DNA binding.[208] A fungal

material extracted from plant samples collected in the Archbold Biological Station (Florida, USA), showed a potent activity against STAT3. The active ingredient of the fungal extract was isolated, characterized and identified as Phaeosphaeride A (**73**). Also the inactive diastereomer Phaeosphaeride B (**74**) was isolated and characterized. Phaeosphaeride A inhibits STAT3 with an IC_{50} of 0.61 μM in the ELISA-based screen. Moreover, Phaeosphaeride A (**73**) induced inhibition of cell growth with an IC_{50} of 6.7 μM. The low micromolar activity may be due to the interaction with another target in the cell, confirmed by testing Phaeosphaeride A (**73**) using STAT3 independent cell lines.



Flavopiridol (**75**)

In the context of the identification of small molecule inhibitors of STAT3, recent studies by Lee *et al.* investigated the hypothesis that an alternative mode of action of potential experimental drug Flavopiridol (**75**) is important cytotoxic agent evaluated in phase I and II clinical trials.[209,210]

Experimental data initially indicated that flavopiridol inhibits cyclin-dependent kinases[211] inducing apoptosis in human cancer cell lines.[211,212] Based on their experimental evidence, *Lee et al.* reported flavopiridol ability to disrupt STAT3/DNA interaction and attenuate STAT3-directed transcription.

3.5 Conclusion

Given the general difficulty and the novelty of STAT3 as target and the importance of the progress made in the identification of small molecules inhibitor of STAT3, this field of drug-discovery is likely to receive increased attention in the future and to stimulate challenging and competitive research.

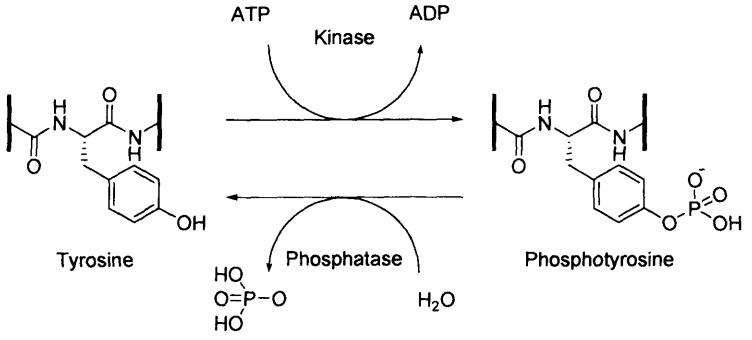
Chapter 4

4.0 Protein phosphatase SHP-2 as anticancer target

4.1 Introduction

Reversible tyrosine phosphorylation is a key mechanism by which signaling pathways are governed and regulated in eukaryotic cells. Protein tyrosine phosphatases (PTPs), which catalyse protein dephosphorylation, and tyrosine kinases (PTKs), responsible for phosphorylation, function as modulators of tyrosine phosphorylation (Scheme 1).

Scheme 1. Phosphorylation and dephosphorylation of tyrosine.



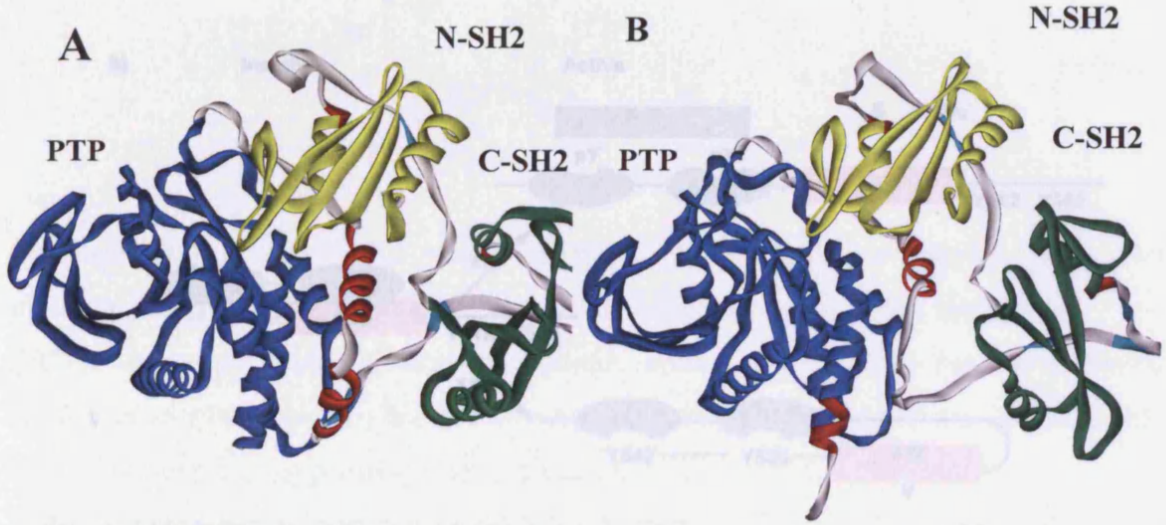
It is well known that abnormal PTK activity due to mutations or overexpression results in oncogenic transformation, and inhibition of tyrosine kinase activity is now established anticancer therapy.[213] While some PTPs have been shown to act as tumour suppressors,[214] it has become more and more evident that deregulation of some tyrosine phosphatases activity is associated with tumourgenesis in different types of cancer. The src homology 2 (SH2) domain containing tyrosine phosphatases SHP-2 is a protein-tyrosine phosphatase implicated in pathogenesis of human diseases including Noonan syndrome (NS),[215] Leopard syndrome,[216,217] juvenile myelomonocytic leukemia (JMML),[218-220] and some adult leukemias.[221] The emerging oncogenic role of SHP-2 has led to its consideration as novel target for anticancer therapy offering the prospect of its pharmacological inhibition. The discovery of small molecule inhibitors of SHP-2 activity has become a topic of great interest in our research in order to provide pharmacologic agents and molecular probes for evaluation and validation of SHP-2 as therapeutic target and for chemical biology studies of its function and signaling mechanism.

4.2 The structural and functional characteristics of SHP-2[222]

The SH2 domain-containing PTPs (SHPs) are small, highly conserved subfamily of cytosolic protein-tyrosine phophatases containing two types of domains that can bind phosphotyrosine

— SH2 and PTP. There are two SHPs present in vertebrates — SHP-1 and SHP-2. The structures of both SHP-1 and SHP-2 have been determined (Figure 23).[223,224] SHPs have two SH2 domains at its N-terminus (N-SH2 and C-SH2), a central protein-tyrosine phosphatases (PTP) domain and a C-terminal tail containing two tyrosine-phosphorylation sites. SHP-2 also has a proline-rich domain.

Figure 23. A) A schematic drawing showing the secondary structure and organization of the domains in SHP-2. The N- and C-SH2 domains are in yellow and green; the catalytic PTP domain is blue. B) Schematic drawing showing the secondary structure and organization of the domains in SHP-1. The N- and C-SH2 domains are in blue and green; the catalytic PTP domain is red.

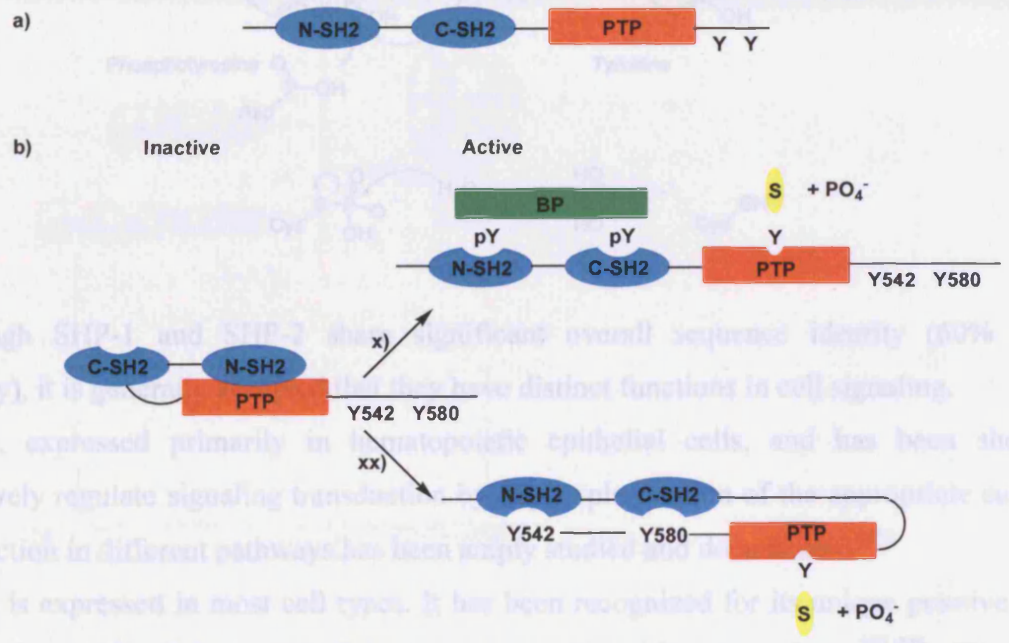


Structural studies have suggested[223] a model for SHPs catalytic regulation. SHPs have a low basal catalytic activity because of the "closed" domain rearrangement in which the two SH2 domains contour around the phosphatase domain. The N-SH2 domain is wedged into and binds to the PTP domain[223] resulting in a "mutual allosteric inhibition" (the N-SH2 inhibits the PTP domain and the PTP domain contorts the Tyr-P binding pocket of the N-SH2 on the opposite surface). The C-SH2 does not interact with the PTP domain and its conformation is left unperturbed (Figure 24).

Interaction of the SHP-binding ligands (bisphosphorylated peptides containing two phosphotyrosine residues — one that can bind the C-SH2 and another that can bind the N-SH2) activates the enzyme. The phosphopeptide binding site of both SH2 domains are exposed on the surface of the molecule, away from the phosphatase domain. The C-SH2 first binds to one of the Tyr-P residues of the bisphosphorylated ligand providing the binding energy and increasing the concentration of the ligand for the N-SH2, which functions as an allosteric switch. Binding of the N-SH2 to the second site of the ligand results in a conformational change and resolves the inhibitory interaction of the N-SH2 and the PTP

domain[222] (Figure 24). A second regulatory mechanism proposed for SHPs activation implies the intramolecular binding of phosphorylated Tyr542 and Tyr 580 of the C-terminal tail to the N-SH2 and C-SH2 respectively. Once activated, SHPs recruit and dephosphorylate their substrate (Figure 24).[222]

Figure 24. a) Schematic representation of SH2 and PTP domains, and C terminal tail of the SHPs. b) Potential mechanism of SHPs regulation. x) In the first mechanism SHP is activated by a SHP-binding protein (BP) containing two phosphotyrosine residues (pY). xx) SHP activation via intramolecular binding of phosphorylated C-tail residues.

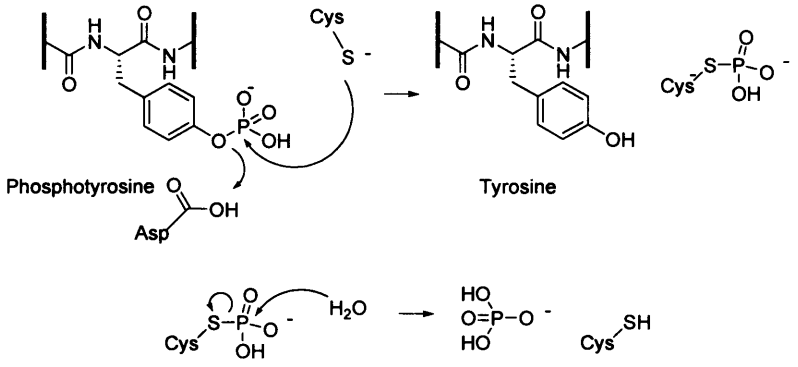


The catalytic or PTP loop (also known as signature motif) is a structural feature highly conserved among the PTPs. The PTP loop comprises 11 residues [(I/V)HCxAGxxR(S/T)G] (x = any aminoacid). Key conserved features of the SHP-2 PTP catalytic cleft include Cys459, the catalityc nucleophile. Several mainchain amide groups in the PTP signature motif form the phosphate binding cleft, which, together with Arg465, hydrogen-bond to the phosphate oxygens of bound substrate. The binding pocket is also characterized by the closure of the so-called "WPD loop" upon ligand binding. On the near WPD loop there are key residue such as Asp425, that functions as a genaral acid in the catalysis.[223]

SHP-2 is a cysteine-dependent phosphatase (CDPs). It catalyses the hydrolysis of a phosphoester bond via a phospho-cysteine intermediate (Figure 25). When the substrate enters the binding site, conformational changes occur in the WPD loop. The loop closes over the phenyl ring of the tyrosine residue, holding and positioning so that the nucleophilic attack occurs. The free cysteine nucleophile forms a bond with the phosphorus atom of the

phosphate moiety, and the P-O bond linking the phosphate group to the tyrosine is protonated by Asp425. This will neutralized the tyrosine, free to diffuse away from the catalytic pocket. The phosphatase is removed from the cysteine via a nucleophilic attack of a water molecule, thus regenerating the active site for another dephosphorylation reaction.

Figure 25. Mechanism of tyrosine dephosphorylation by SHP-2.



Although SHP-1 and SHP-2 share significant overall sequence identity (60% overall identity), it is generally accepted that they have distinct functions in cell signaling.

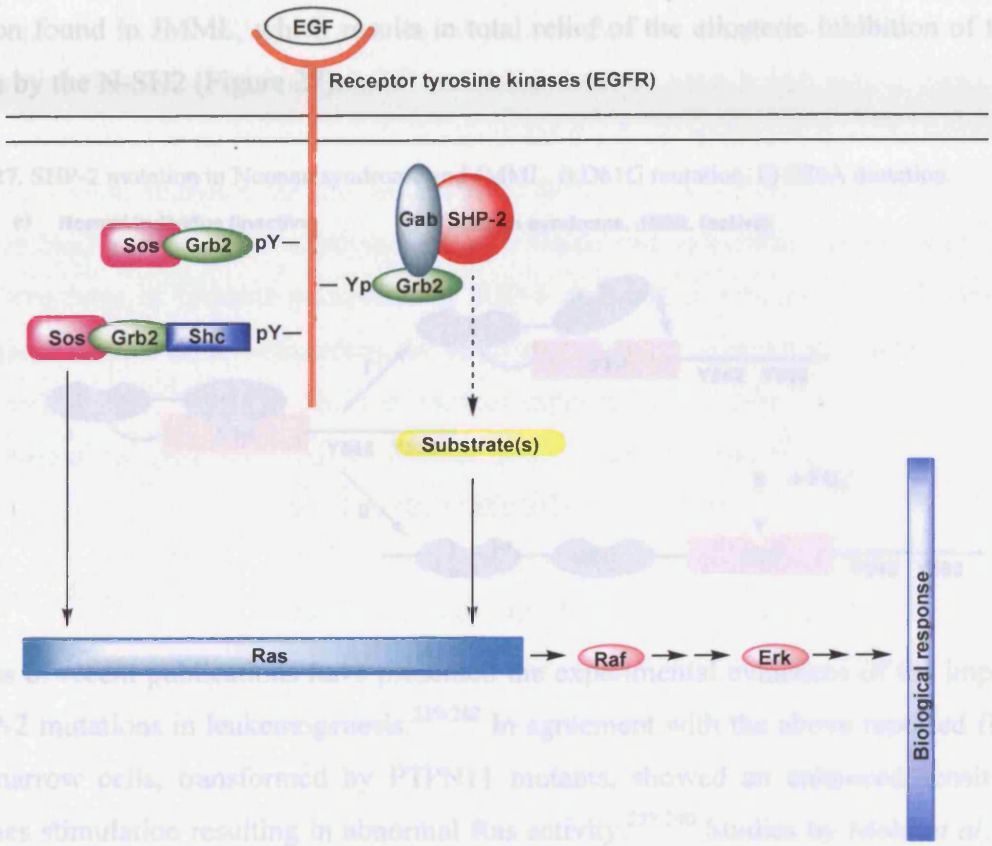
SHP-1, expressed primarily in hematopoietic epithelial cells, and has been shown to negatively regulate signaling transduction by dephosphorylation of the appropriate substrate. Its function in different pathways has been amply studied and documented.[222]

SHP-2 is expressed in most cell types. It has been recognized for its unique positive role in regulation of Ras-Erk (extracellular signal-regulated kinase) pathway[222,225] (Figure 26) stimulated by epidermal growth factors and cytokines. Therefore, SHP-2 functions as a positive regulator of cell proliferation.[226-229] Ras protein belongs to the superfamily of small guanosine triphosphate hydrolases (GTPases) and is considered a crucial node for signaling routes controlling cell proliferation, differentiation, survival, migration, and metabolism.

Ras activation is induced by growth factors receptors acting through receptor tyrosine kinases (RTKs) (Figure 26). It is well understood that multiple pathways can lead to Ras activation. Following growth factor (GR) stimulation, RTKs trans-autophosphotylate on specific tyrosine residues, creating a binding site for diverse docking proteins containing SH2-domain.[225] These include the proteins Shc and Grb2 and Gab1. Mechanism of Gab1 activation involves phosphorylation on specific tyrosine residues. Once phosphorylated, Gab1 recruits a series of SH2 domain-containing proteins that include SHP-2. Cunnick and coworkers reported that phosphotyrosine 627 and 659 of Gab1 constitute a bisphosphoryl tyrosine-based activation motif for binding and activation of SHP-2.[230] Moreover, a set of independent data suggested

that Gab1-SHP-2 interaction and SHP-2 PTP activity are necessary for Ras-Erk pathway activation by growth factors and cytokines.[222,230-234] To date, SHP-2 function upstream from Ras has not elucidated and the biochemical model illustrating the physiological substrates linking SHP-2 to Ras remain to be established.

Figure 26. Model for Ras pathway activation during EGF stimulation.



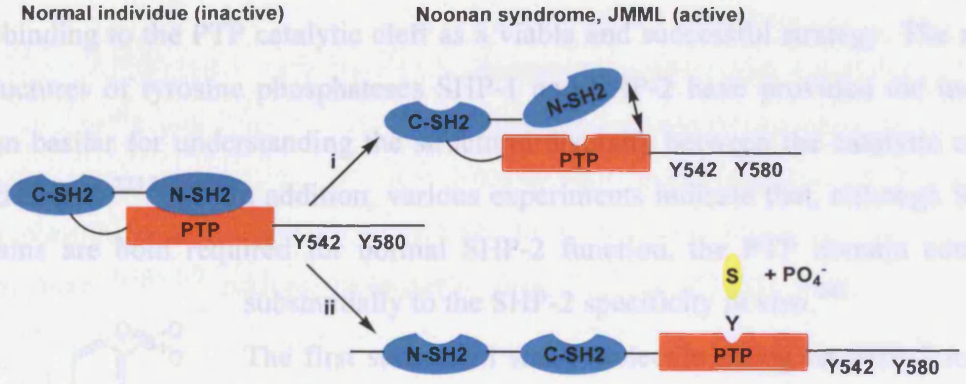
4.3 SHP-2 in human cancer and diseases

Implication of SHP-2 mutations in several human diseases has been amply documented. Mutations of the human gene PTPN11, encoding SHP-2, have been detected in patients with juvenile (JMML) and childhood leukemias[218,219,235] (the major Shp2-asssociated malignancy), Leopard Syndrome,[216,217] Noonan Syndrome (NS), a developmental disorder characterized by an abnormal face, webbed neck, proportionate short stature and cardiac abnormalities. Overexpression of SHP-2 appears to be implicated in leukemogenesis in adult human leukemia.[221] SHP-2 has been also associated with pathogenesis by *Helicobacter pylori*, the major cause of gastric ulcer and carcinoma worldwide.[236,237] In gastric epithelial cells, the *H. pylori* virulence determinant, CagA protein, becomes tyrosyl phosphorylated, and recruits and

activates SHP-2. Experimental evidences suggest that SHP-2 recruitment is indispensable for CagA ability to induce transformation of gastric epithelial cells.[237]

Many of the disease-associated SHP-2 mutations effect the N-SH2/PTP domain interface and were demonstrated to disrupt the "mutual allosteric inhibition" between the N-SH2 and PTP domain and lead to SHP-2 activation.[238] Impairing the N-SH2/PTP inhibitory interaction results in an "activated" SHP-2 protein and increased basal activity. E76A is a severe mutation found in JMML, which results in total relief of the allosteric inhibition of the PTP domain by the N-SH2 (Figure 27).

Figure 27. SHP-2 mutation in Noonan syndrome and JMML. i) D61G mutation. ii) E76A mutation.



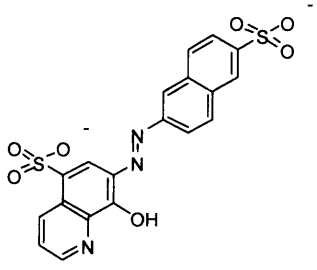
A series of recent publications have presented the experimental evidences of the implication of SHP-2 mutations in leukemogenesis.[239-242] In agreement with the above reported findings, bone marrow cells, transformed by PTPN11 mutants, showed an enhanced sensitivity to cytokines stimulation resulting in abnormal Ras activity.[239,240] Studies by Mohi *et al.* proved that, in order for SHP-2 mutants to induce aberrant Ras activation, Gab2/SHP-2 interaction is required.[239] Together all these data and findings provide more evidences of PTPN11 oncogenicity and further support SHP-2 as therapeutic target for treatment of cancer.

4.4 SHP-2 inhibitors as potential anticancer drugs

SHP-2 represents an ideal candidate target for anticancer drug development as result of the discovery of its positive regulation of the Ras/Erk signaling pathway and the identification of oncogenic mutations of SHP-2. The development of PTP inhibitors as potential anticancer drugs has gained more and more interest due to the increasing number of protein-tyrosine phosphatases with a proven or potential oncogenic role.[214,243] Significant and successful efforts have been made to identify potent and selective PTP inhibitors. The pool of natural products has always represented a precious source of compounds, but the increasing number

of crystallographic data available has provided the tools for the structure-base design of inhibitors.[243] The identification of small molecules inhibitors provides powerful tools to determine whether protein tyrosine phosphatases are suitable candidates for drug development, or, in other words, to verify their "druggability". Due to the novelty of the research field, potent inhibitors are lacking for SHP-2 and a few examples are reported in the literature. Cell penetrating small molecules would be extremely valuable reagents to probe the biological function of SHP-2 and would provide rational design parameters for potential inhibitors. A detailed understanding at molecular level of SHP-2 and SHP-1 catalysis and substrate specificity is an indispensable requisite for the development of specific inhibitors.

However, several studies offer the molecular basis for the development of selective SHP-2 inhibitors binding to the PTP catalytic cleft as a viable and successful strategy. The reported crystal structures of tyrosine phosphateses SHP-1 and SHP-2 have provided the molecular information basilar for understanding the structural diversity between the catalytic cavity in SHP-2 and SHP-1.[223,224,244] In addition, various experiments indicate that, although SH2 and PTP domains are both required for normal SHP-2 function, the PTP domain contributes substantially to the SHP-2 specificity *in vivo*.[245]



NSC 87877 (**76**)

The first successful small molecule acting on SHP-2 to inhibit Erk1/2 activation has been recently reported in the literature.[246] Our group with collaboration of Dr Wu identified 8-hydroxy-7-(6-sulphonaphthalen-2-yl)diazenyl-quinoline-5-sulfonic acid (NSC87877) (**76**) as a potent SHP-2 inhibitor by screening the National Cancer Institute Diversity Set.

NSC87877 **76** potently inhibited SHP-2 (IC_{50} = 0.318 ± 0.049 μM) selectively over diverse PTPs, but it seemed to have no selectivity between SHP-2 and SHP-1 (IC_{50} = 0.355 ± 0.073 μM).

In the design or identification of SHP-2 inhibitors the selectivity problem needs to be addressed. Several hits from the NCI Diversity Set, displayed selectivity between SHP-1 and SHP-2, indicating that development of SHP-2 selective inhibitors should be possible. Computational studies also suggested that compound **76** binds to the SHP-2 PTP domain. Computer docking indicated that the B-ring sulfonic acid group forms a hydrogen bond with the NH-group of Arg465 residue located at the base of the PTP domain[247] (Figure 28).

The A-ring sulfonic acid forms hydrogen bonds with the side-chain NH_3 group of Lys280 and the side-chain NH_2 group of Asn281, PTP amino acid residues.[247] The involvement of Lys280 and Asn281 in NSC87877 binding to SHP-2 was supported by further docking

studies involving SHP-2 mutants SHP-2V280 and SHP-2RD (containing changes in the Lys280 and Asn281). An increase in the docking scores was observed. Compound **76** lower binding affinity for SHP-2 mutants was confirmed by increased IC_{50} values *in vitro* experiments (SHP-2V280 $IC_{50} = 1.110 \pm 0.136$ μM, SHP-2RD $IC_{50} = 1.087 \pm 0.162$ μM).

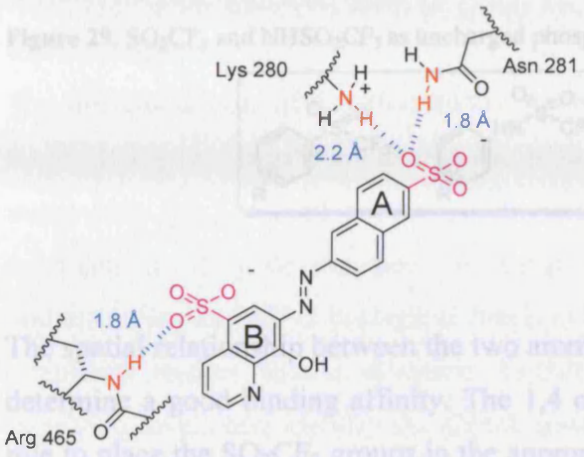


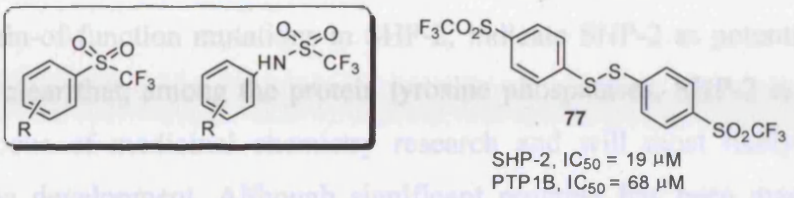
Figure 28. Molecular model of **76** binding to the SHP-2 PTP domain.

The aryl sulfonic acid group has been previously identified as pharmacophore of various PTP inhibitors *in vitro*.[248,249] It appears to interact with the PTP catalytic site acting as a mimic of phosphate group in pTyr. Numerous and selective PTP inhibitors containing phosphate mimicking moieties have been reported. These include phosphonic acids derivatives,[248,250-252] malonic acid derivatives,[248,253-255] sulfonic acids,[256,257] cinnamic acids,[248,258,259] tetrazole,[248,256] and oxamic acids.[248,260,261] In the development of PTP inhibitors, the naphthyl-substituted or biphenyl compounds generally show the highest potency because of the role played by hydrophobic interactions in the binding to the active site.[243] The hydroxyl group and the quinoline moiety may be critical for activity. Further structure optimization could be conducted by replacing the diazo bond with its stable bioisosters such as sulfonamide and amide bond. The diazo bond represents a structural shortfall. It can be easily metabolized in the human body by enzymes generating the corresponding aniline. This could lead to loss of activity or, perhaps, to an increased toxicity due to the properties of the metabolites.

Unfortunately, the phosphate mimics constitute often highly-charged components of the molecules and, due to the polarity, many PTP inhibitors lack membrane permeability limiting their therapeutic utility. Huang and coworkers[248] described a structure-base design of PTP1B phosphatase inhibitors which incorporates a phosphate mimic group, an aromatic ring imitating the aryl ring in the pTyr, and additional structural motifs that could enhance affinity and selectivity by interacting with the enzyme surface. Among the selected compounds subjected to docking studies, bis(4-trifluoromethylsulfonylphenyl)disulfide (**77**) was chosen as lead candidate. The lead optimization led to the identification of suitable linkages to the replace the metabolically labile disulfide bond and indicated the easily chemically accessible

trifluoromethyl sulfonamide group (—$NHSO_2CF_3$) as further neutral phosphate mimic (Figure 29). Biochemical screening results showed that compound **77** (Figure 29) and its derivatives **78** (Figure 30) have a significant inhibitory activity toward PTP1B and other phoshpateses including SHP-2 (Figure 29).

Figure 29. SO_2CF_3 and $NHSO_2CF_3$ as uncharged phosphate mimic. Lead compound **77**.

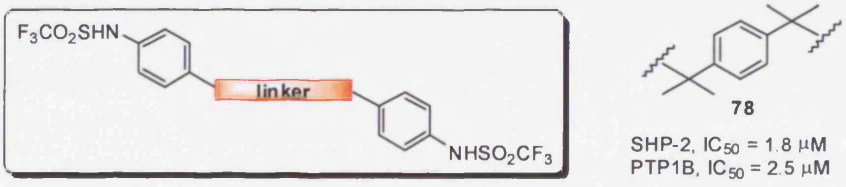


The spatial relationship between the two aromatic rings appeared to be an important feature to determine a good binding affinity. The 1,4 or 1,3-di-substituted phenyl linkages have a key role to place the SO_2CF_3 groups in the appropriate position to maximize the interactions with the aminoacid residues resulting in a significant increase of the activity as shown by compound **78** (Figure 30). Moreover, further SAR indicated that both SO_2CF_3 are essential for good activity (data not shown).

The two-site-binders constitute a well-explored template for development of PTP selective inhibitors.[243] This approach is based on the discovery of presence of a second aryl-phosphate-binding site adjacent to the active site. This second site is less conserved among PTPs.[262] Bidentated ligands binding the two sites may exhibit an enhanced activity and selectivity.

The trifluoromethyl sulfonyl and trifluoromethyl sulfonamides **78** possess a remarkable structural similarity to **76**. It is tempting to suggest that they share a similar mode of action, although crystallography studies and NSC87877-based SAR studies would be necessary to verify the hypothesis.

Figure 30. —SO_2CF_3 compound **78**.



Finally, targeting the SH2 domain has been suggested as feasible and useful option to achieve selectivity.[214] The blockade of the SH2 domain-dependent protein-protein interaction has

become a valuable strategy to prevent phosphorylated activated receptors from binding the SH2 domain of signaling partners.[263,264]

Unfortunately, early SHP1 and SHP-2 inhibitor designs relied on peptidic structures[265,266] and there are no small molecules ligands of the SH2 domain reported in the literature.

4.5 Conclusion

The oncogenic gain-of-function mutations in SHP-2, indicate SHP-2 as potential anticancer target. It appears clear that, among the protein tyrosine phospatases, SHP-2 is beginning to move into the focus of medicinal chemistry research and will most likely be a prime candidate for drug development. Although significant progress has been made toward an understanding of SHP-2 biological function its involvement in the development of diseases, continued studies should elucidate further its role in signal transduction pathways at satisfying levels and identify the signal downstream from SHP-2. Small molecule inhibitors are much needed as valuable tools for chemical and biological studies of SHP-2 function and validation of SHP-2 as a therapeutic target.

Chapter 5

5.0 Aurora kinases as targets for anticancer drug development

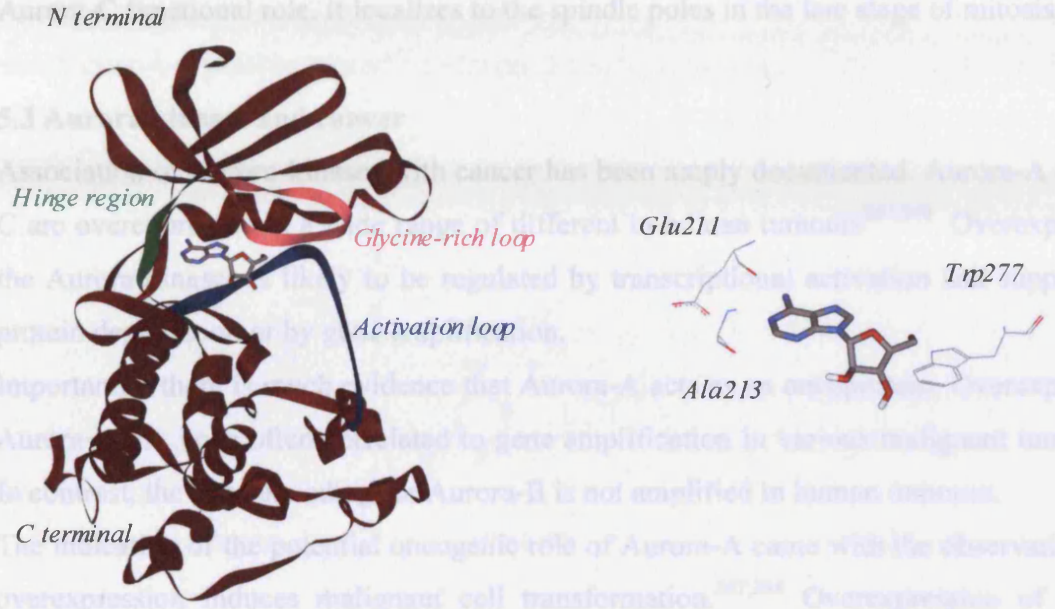
5.1 Introduction

In cellular division progression through M-phase is controlled by phosphorylation events performed by several serine/threonine kinases, known as mitotic kinases. Among this network of regulatory proteins, the three human homologues of Aurora kinase (A, B, and C) have been amply investigated as they are essential for the execution of numerous mitotic events and required for genome integrity and stability. Studies have shown that Aurora A and B are frequently overexpressed in various cancer cells when compared to adjacent normal tissue, indicating their implication in cancer.[267] Aurora-A also acts as an oncogene and induces malignant transformation when overexpressed.[267] In recent years there has been great interest in developing small molecules inhibitors of Aurora kinases as potential novel anticancer drugs.

5.2 Biological roles of the Aurora Kinases[268-271]

Proteins kinases are enzymes that catalyse the transfer of orthophosphate residue (PO_3^-) from adenosine-5'-triphosphate (ATP) to hydroxyl group of a specific amino acid residue of their substrate targets. Protein kinases are classified, based on their substrate specificity, as serine/threonine and tyrosine kinases. The Aurora kinases comprise a family of serine/threonine kinases consist of three members — A, B and C — in vertebrate species. They participate in and regulated different mitotic events.[272] The overall homology between the three Auroras in human is about 60% at amino acid level, with their highly conserved N-terminal catalytic domain and a short C-terminal.[273] These two domains are linked together by a hinge region. The crystal structure of the C-terminal catalytic domain of Aurora-A bound to adenosine has been recently determined (pdb1muo) (Figure 31) by X-ray diffraction.[274] The adenosine residue binds in a deep hydrophobic cleft at the interface between the C- and N-terminal domains. Residues Glu211 and Ala213 of the hinge region of the kinase specifically hydrogen bond to the purine ring of adenosine (Figure 31). The side chain of the residue Trp277, located in the activation loop, binds to adenosine through specific hydrogen bonds (Figure 31). The active site cleft is bounded by a glycine-rich loop, which contains a consensus kinase sequence (Gly-x-Gly-xx-Gly) and the activation loop. The purine ring of adenosine is positioned between the residue Leu263 and the hydrophibic surface of the glycine-rich loop (Leu137, and Val147) and Ala 160. Thr288, which is phosphorylated during the activation of Aurora-A is located in the activation loop.

Figure 31. The overall structure of the Aurora-A adenosine complex[274]. The hinge region, the glycine-rich loop, and the activation loop are coloured in green, pink, and blue, respectively. Residues Glu211 and Ala213 of the hinge region of the kinase specifically hydrogen bond to the purine ring of adenosine. Trp277, located in the activation loop, binds to adenosine through specific hydrogen bonds.



Although the catalytic domains of the Aurora kinases are highly conserved, the Auroras are characterized by different substrate affinity, subcellular localization and associated activities. Their activities gradually increase at the S-phase to peak at the M-phase. The kinases are degraded by the proteasome upon exit from mitosis.

Aurora-A localizes to the centrosomes and to the spindle poles in mitosis. It is believed to be essential for the regulation of several processes such as building the bipolar spindle, including centrosome maturation and separation. Aurora-A kinase activity is regulated by autophosphorylation of Thr288 during G2/M phase upon interacting with its binding partner TPX2 (a protein playing a key role in spindle assembly), Ajuba, and HEF1.[275-277] Aurora-A phosphorylates several proteins which are important in mitosis, including histone H3 on Ser10.[278,279] Repression of Aurora-A expression by genetic techniques has been shown to delay mitotic entry human cells[276], and its overexpression can compromise the spindle check-points[280] and inhibit cytokinesis.[281]

Aurora-B is a *chromosomal passenger*, a protein localizing to the centromeres in the early phase of mitosis, but relocating to the spindle midzone following anaphase onset. It plays an essential role in chromosome segregation and cytokinesis. Aurora-B activation is triggered by authophosphorylation after association with its substrates INCENP and survivin.[282,283] During

mitosis, Aurora-B is responsible for the phosphorylation of histone H3 on both Ser10 and Ser28 and of centromere protein A (CENP-A) on Ser7.[284,285] These modifications appear to be required for proper chromosome dynamics during mitosis. Aurora-B kinase activity is also required for bipolar chromosome orientation and condensation. Little is known about Aurora-C functional role. It localizes to the spindle poles in the late stage of mitosis.[286]

5.3 Aurora kinases and cancer

Association of Aurora-kinases with cancer has been amply documented. Aurora-A and B and C are overexpressed in a wide range of different in human tumours[267,268]. Overexpression of the Aurora kinases is likely to be regulated by transcriptional activation and suppression of protein degradation or by gene amplification.

Importantly, there is much evidence that Aurora-A acts as an oncoprotein. Overexpression of Aurora-A has been often correlated to gene amplification in various malignant tumour.[271,287] In contrast, the gene encoding for Aurora-B is not amplified in human tumours.

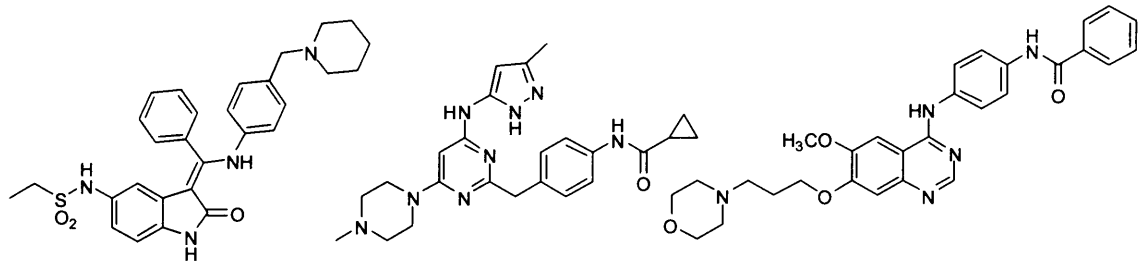
The indication of the potential oncogenic role of Aurora-A came with the observation that its overexpression induces malignant cell transformation.[267,288] Overexpression of Aurora-A induces disruption of cell-cycle checkpoints function such as the DNA-damage-induced G2 checkpoint[271,289] and tetraploidization.[271] Tetraploidization is a precursor of aneuploidy, an abnormality in gene copy number, which is the most prevalent cell genomic alteration identified in tumours,[290] and related to cancer development.[291] Normal cells have a checkpoint (known as post-mitotic G1 checkpoint) that induces G1 arrest when cells become tetraploid. When Aurora-A is overexpressed in cells lacking the post-mitotic G1 checkpoint, tetraploid cells continue their cell cycle acquiring extra centrosomes and further chromosome instability. Aurora A is also mutated in certain cancers. It has been recently reported a correlation between Aurora-A and the tumour suppressor p53, suggesting its real connection to oncogenesis. Although only Aurora-A is considered an oncogene, cells overexpressing Aurora-B induced aggressive tumour and metastasis when implanted in nude mice.[292,293] Aurora-C function in tumourgenesis is presently unclear.

5.4 Aurora kinases inhibitors

In view of their implication in tumorgenesis, the Auroras are promising targets for anticancer drug development. Disruption of their ability to interact with their binding partners could be one potential strategy to inhibiting their function. However, targeting protein-protein interaction has been demonstrated to be problematic and difficult to achieve. The Auroras are

amenable to small molecule inhibition by targeting the kinase activity at the ATP binding site. This has been proven to be a feasible and successful approach. Inhibition of Aurora kinases activity disrupts the cell cycle and block proliferation. Many inhibitors are now available. Hesperadin[294] (**79**), VX680[295] (**80**), and ZM447439[296] (**81**) (Figure 32) represent the first generation small molecules inhibitors of Aurora kinases. They were designed from the Aurora-A crystal structure[274] to target the catalytic domain of the kinases and occupy the ATP binding-site.

Figure 32. First generation small molecules inhibitors of Aurora kinases.



Due to the fact that the catalytic domain of the three kinases is highly conserved, these drugs were expected to inhibit all three Auroras. Given the oncogenic role or the overexpression of the three kinases in tumours, targeting the all the members of the Auroras family might not interfere with the therapeutic success. These first-generation small molecule inhibitors showed sufficient selectivity for Aurora family to analyze the phenotype deriving from inhibition of these enzymes. Hesperadin (**79**) inhibits Aurora-B *in vitro* (IC_{50} value of 0.25 μM), while exhibits more potency (0.02-0.1 μM) in cell based assays.[294] Hesperadin (**79**) has apparently not been tested against Aurora-A. The quinazoline derivative **81**, identified as an ATP competitive inhibitor, *in vitro*, inhibits Aurora-A and B with IC_{50} values of approximately 100 nM.[296] VX680 (**80**) inhibits Aurora-A, B, and C, *in vitro* with inhibition constant 0.6, 18, and 4.6 nM, respectively. All three compounds inhibit phosphorylation of histone H3 on serine 10 and cytokinesis.[294-296] However, the cells do not undergo a simple mitotic arrest, the cell cycle proceeds with normal timing, and entry and exit from mitosis are unaffected. This cell cycle progression without resulting cytokinesis induces tetraploid cells. Longer exposures reveal cell-line-dependent effects.[294-296] Treated cells either continue to cycle without cytokinesis and become highly polyploid (and ultimately die undergoing apoptosis) or alternatively undergo cell cycle arrest. Moreover, VX-680 (**80**) displays potent

antitumor activity in mice models, is well-tolerated preclinically, and has subsequently entered clinical development.[295]

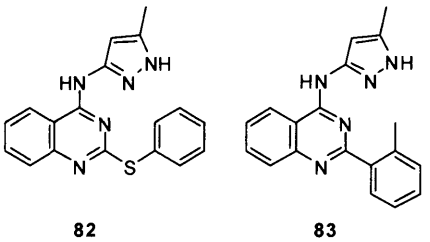
The unique effect on tumour cells, shown by Aurora kinases inhibitors, distinguishes their behavior from that of classic "antimitotic agents", known to lead to mitotic arrest.

The phenotypes deriving from exposure of cells to these three inhibitors appear to be consistent with an inhibition of Aurora-B, and do not resemble the effect reported for inhibition of Aurora-A by genetic means. These observations raised the hypothesis that the kinase activity of Aurora-A may not be important for its activity and may not represent an attractive target for drug discovery. Its biological role in mitosis is independent of its kinase activity.

It could be beneficial to develop selective inhibitors of a particular Aurora kinase, rather than inhibitors of the whole family in order to elucidate which Aurora is responsible for the antitumour activity after drug treatment.

Development of a number of different chemical classes of compounds inhibiting one or more of the Aurora kinases have been reported in the literature. The majority of the medicinal chemists efforts have been initially focused on the inhibition of the Aurora-A as the most desirable mode of action.

VX680 (**80**) is a 4,6-di-aminopyrimidine emerged as Vertex's most promising compound from a program aimed at identification of small molecules inhibitors of Aurora kinases targeting the ATP-binding site. Vertex has also published a number of patent applications describing novel series of protein kinases inhibitors displaying the highest potency for Aurora-A, GSK3 and Src kinases[297-303]



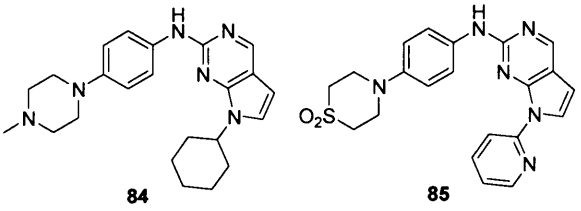
82

83

Figure 33. Structures of VX680 (**80**) analogues.

Analogues of VX680, based on the pyrimidinylaminopyrazole-scaffold have been designed and synthesized. Interesting examples emerged from SAR studies, are compounds **82** and **83**, which have an inhibitory constant < 0.1 μM for Aurora-A and 0.1-10 μM for GSK3 and Src kinases. (Figure 33)

Independent research conducted at Johnson and Johnson led to the identification of a new class of Aurora-A inhibitors based on pyrrolopyrimidine **84** (Figure 34).[304] From *in vitro* screening of a compound collection, **84** emerged for its excellent level of Aurora-A enzyme inhibition (IC_{50} 0.047 μM) as a potential lead compound for further development.

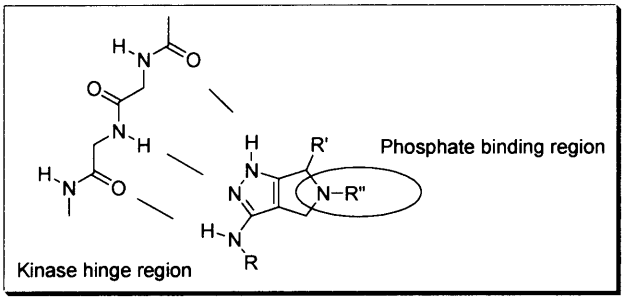


84 85

Figure 34. Structure of pyrrolopyrimidine derivatives.

The synthesis and SAR led to the optimized analogue **85** (Figure 34) possessing sub-nanomolar *in vitro* potency (IC_{50} 0.0008 μM), good kinase selectivity showing 90-, 250-, 389-, >1000-, 163-, >1000-, and >1000-fold selectivity for Aurora-A over CDK1, IRK Src, PDGF, c-Met, Plk1, and FAC kinases, respectively. Aurora-B data have been not reported. Moreover, compound **85** inhibited the histone-H3 phosphorylation on Ser10 (which is regarded as a marker of Aurora-B inhibition), and exhibited anti-proliferative activity against several tumour cell lines in the range.

Novel and potent Aurora-A and CDK2 kinases inhibitors have been designed based on the 3-amino-tetrahydropyrrolo[3,4-*c*]pyrazole scaffold, a versatile scaffold designed to target the ATP binding pocket of protein kinases. In particular, the 3-aminopyrazole moiety forms hydrogen bonding interactions with the kinase hinge region of the ATP pocket, whereas the 5-substituent appears to enter the phosphate binding region[305] (Scheme 2)



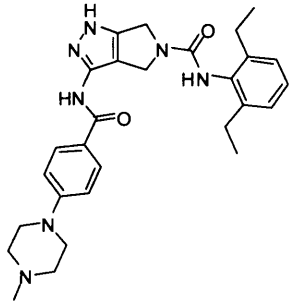
Scheme 2. Schematic representation of the – amino tetrahydropyrrolo[3,4-*c*]pyrazole scaffold in the kinase ATP binding pocket.

Development of the pyrazole series led to the discovery of compounds PHA680632[305] (**86**), and compound[306] **87**, displaying high potency for Aurora-A and improved selectivity profile toward the inhibition of Aurora-A (Figure 35).

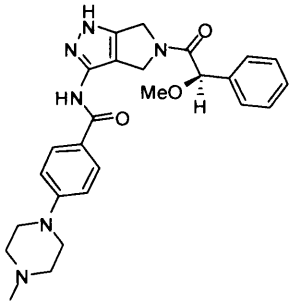
The X-ray crystal structure of **86**, solved with the kinase domain of Aurora-A, showed the expected hydrogen bonding interaction of the 3-aminopyrazole with the kinase hinge region, while the diethylphenylurea group is directed approximately perpendicular to the pyrrolopyrazole. Cell based assays identified compound **86** as a potent Aurora kinase inhibitor, able to block cell cycle progression and inhibit histone H3 phosphorylation on Ser10 (which is regarded as a marker of Aurora-B inhibition) in HCT-116 cells (antiproliferative IC_{50} = 0.045 μM). Moreover, **86** displayed an antiproliferative effect (antiproliferative IC_{50} in the nanomolar range) on a wide range of different cell lines. In the effort to optimize and increase the inhibitory potency in this series, Fancelli and coworkers

synthesized a small library of 5-phenylacetyl 1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazole derivatives, leading to the identification of compound **87**.

Figure 35. Structures and *in vitro* SAR of 3-amino-tetrahydropyrrolo[3,4-*c*]pyrazole series.



86, IC_{50} (Aurora-A) = 0.027 μM
IC_{50} (Aurora-B) = 0.135 μM
IC_{50} (Aurora-C) = 0.120 μM

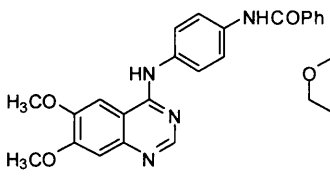


87, IC_{50} (Aurora-A) = 0.013 μM
IC_{50} (Aurora-B) = 0.079 μM
IC_{50} (Aurora-C) = 0.061 μM

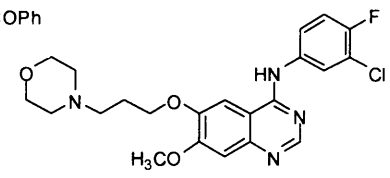
This compound displays a remarkable Aurora-A and B inhibitory activity (Figure 35). It exhibited a potent antiproliferative effect on different cancer cell lines (IC_{50} value between 28-140 nM) and induced a complete suppression of histone H3 phosphorylation on Ser 10. Finally, **87** appeared to be efficacious in a range of tumour models. The binding mode was revealed by the X-ray crystal structure of Aurora-A solved in complex with **87**. Based on the favorable *in vitro* and *in vivo* profile, compound **87** has been selected for evaluation as a potential anticancer agent and is currently under investigation in Phase I clinical studies.

A series of anilinoquinazolines, exemplified by compound **88**, emerged as potent and selective Aurora kinase inhibitors from the high throughput screening of the AstraZeneca compound collection (Figure 36).

Figure36. Structures of kinase inhibitors developed by AstraZeneca.



88



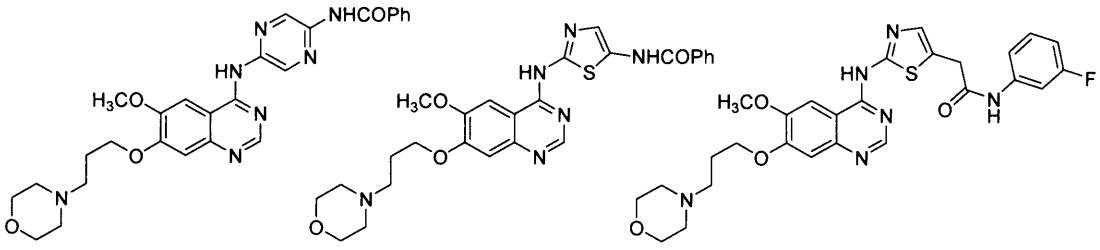
Iressa™ (**89**)

Compound **88** exhibits remarkable strucutures similarity with Iressa™ (**89**) (marketed by AstraZeneca), the first selective inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinase. Iressa functions by binding to the adenosine triphosphate (ATP)-binding site of the enzyme, and, based on X-ray structural data, the quinazoline moiety binds into the

adenine site with the N-1 making a critical key interaction with the protein backbone. The same binding mode was expected and hypothesized for **88**.

The lead optimization of **88** was firstly directed to the replacement of the aniline core group with a range of six and five-membered heterocycles, conferring more hydrophilicity and increased potency to the second generation analogues. The 5-pyrimidine analogue **90** achieved an increased inhibitory activity, exhibiting an excellent affinity for the Aurora kinases (Figure 37), and, attributable to a reduced lipophilicity, an improved plasma protein binding (data not shown). Compound **90** was also active in an MCF7 cellular proliferation assay (IC_{50} = 0.210 μM).

Figure 37. *In vitro* Aurora kinase inhibition of 6-memberd ring heterocycles analogues of compounds **88**.



90, Aurora-A IC_{50} 0.003 μM
Aurora-B IC_{50} 0.001 μM

91, Aurora-A IC_{50} 0.004 μM
Aurora-B IC_{50} 0.042 μM

92, Aurora-A IC_{50} >0.001 μM
Aurora-B IC_{50} >0.001 μM

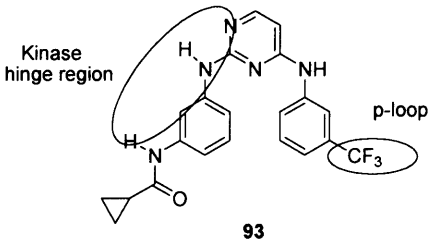
A series of analogues, containing five-membered heterocycles as novel linking groups, was prepared and evaluated for inhibitory potency.[307-310]

The excellent affinity for Aurora kinases showed by compound **91**, indicated that the substitutions were well tolerated, and suggested that the kinases have some flexibility in the selectivity pocket.

Further comfomational changes were introduced in the third generation library. The introduction of an extra methylene group between the amide carbonyl and the five-membered heterocycle ring gave compound **92** (Figure 37) with an increased potency as compared to the parent compound **88**.[308-310]

In a medicinal chemistry program aimed at the discovery of potent and selective inhibitors of EGFR,[311] cyclopropanecarboxylic acid-(3-(4-(3-trifluoromethylphenylamino)-pyrimidin-2-ylamino)-phenyl)-amide (**93**) displayed a weak EGFR inhibitory activity (IC_{50} > 1000 μM), while strongly emerged as potent Aurora-A (IC_{50} > 42 nM).[312] The 2,4-anilinopyrimidine derivatives have been investigated as potent inhibitors of tyrosine and serine/threonine kinase inhibitors.[313-315] The crystal structure of Aurora-A complexed with **93** revealed a novel

binding mode for the 2,4-dianilinopyrimidine core which could provide the basis for the design of more potent and specific analogs of **93**. In particular, compound **93** adopts an s-cis conformation upon binding to Aurora-A. The dipyrimidine and aniline moieties bind in an adenine mimetic faschion, forming hydrogen bonding interactions with the kinase hinge region of the ATP pocket, whereas the cyclopropylamide is oriented away from the active site and partially solvent exposed. The trifluoromethyl group interacts with the kinase phosphate-loop (p-loop) (Figure 38).

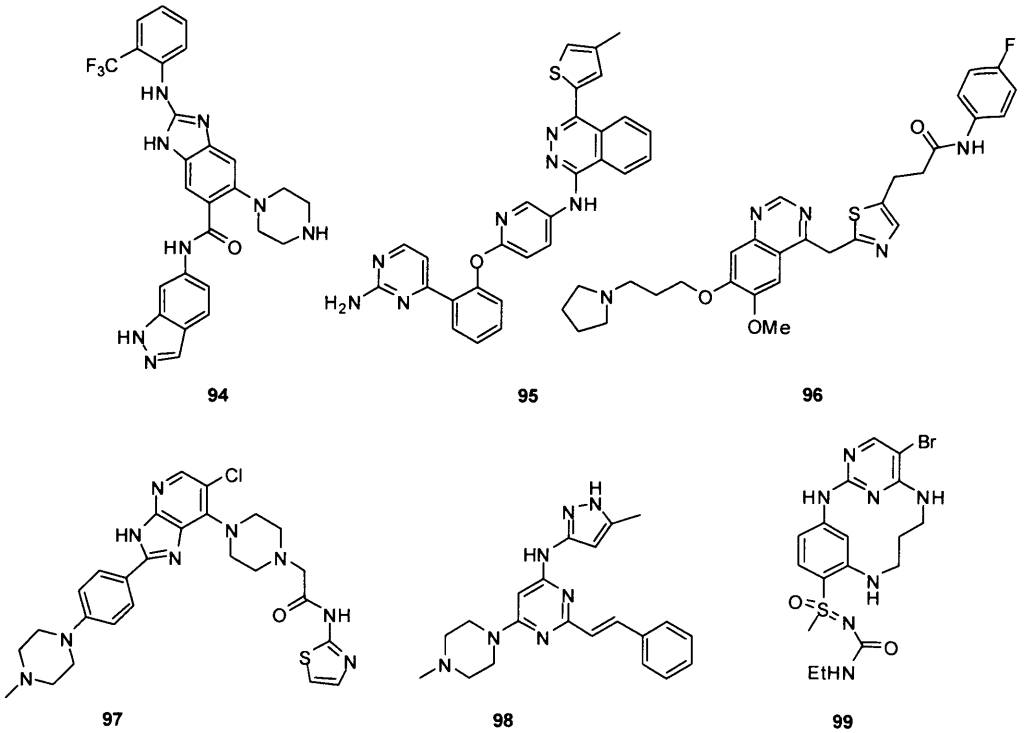


93

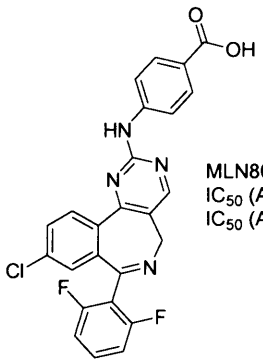
Figure 38. Structure and schematic representation of cyclopropanecarboxylic acid-(3-(4-(3-trifluoromethylphenylamino)-pyrimidin-2-ylamino)-phenyl)-amide (**91**) in the kinase ATP binding pocket.

A review of the recent patent literature for the period 2006 to 2007 revealed how the search for Aurora kinases inhibitors has rapidly progressed as many of the major pharmaceutical companies and academic institutes have shown a great interest in the field[316-321] A few examples of Aurora kinase inhibitors are shown in Figure 39.

Figure 39. Structures of the Aurora kinase inhibitors emerged from the recent patent literature.


94 95 96 97 98 99

In this contest, recent studies conducted at the Millenium Pharmeceutical Inc. laboratories led to the discovery of MLN8054 (**100**) a benzodiazepine derivative, selective inhibitors of Aurora-A kinase.[322] MLN8054 (**100**) exhibited > 40-fold selectivity for Aurora-A over Aurora-B, and >100-fold selectivity over a panel of 226 kinases (data not shown), and also inhibited proliferation of a battery of human cancer cell lines (IC_{50} values range from 0.11 to 1.43 μM). The phenotype emerged from was consistent with the inhibition of Auorara-A by genetic means. Treatment of tumour cells with MLN8054 (**100**) resulted in inhibition of Thr288 autophosphorylation, delays G_2/M progression, resulting in cell death through apoptosis. MLN8054 (**100**) inhibited Aurora-A activity in HCT-116 tumour cells at 0.25 and 1 μM. No inhibition of the phosphorylation of histone H3 on serine 10 was measured or detected, demonstrating that Aurora-B was not inhibited at these concentrations. Finally, MLN8054 (100) exhibited potent antitumor activity in mice models and is currently in Phase I clinical trials.



MLN8054 (**100**)
IC_{50} (Aurora-A) 0.004 μM
IC_{50} (Aurora-B) 0.172 μM

5.5 Specificity

The potential therapeutic use of Aurora kinase inhibition has been based on the hypothesis that in non-proliferating cells (the most normal cells in the human body) Aurora kinases are only expressed and active during mitosis.[323,324] The identification of Aurora inhibitors has provided valuable tools in support of this hypothesis. Ditchfield and coworkers reported that non-proliferating cells were not affected by ZM447439 (**81**) treatment.[325] Moreover, tumour-growth inhibition or regression has been observed in nude mice bearing tumour xenografts in response to treatment with VX680 (**80**).[325] VX680 (**80**) has been reported to be more toxic in rat models. At 1mg/Kg/h dose level, neutrophil counts were partially suppressed by, and then returned to normal level when treatments were stopped. This indicated that bone-marrow stem cells, from which the neutrophils are derived, might be a target of this drug. However this is the only reported effect and seems to be reversible.[325]

5.6 Conclusion

Since the association between Aurora kinases and cancer made in 1998, a body of evidence has been acquired on their role in tumourgenesis. Considerable efforts have been dedicated to the identification of Aurora kinases inhibitors to provide pharmacological tools to validate the

Aurora kinases as drugable target in cancer therapy. Based on the encouraging *in vitro* and *in vivo* profiles, small molecule inhibitors of Aurora kinases are currently progressing into clinical trials. In the future, the clinical success of these inhibitors may provide the compelling evidence that the inhibition of the Aurora kinase family represents a new and effective approach for the treatment of cancer.

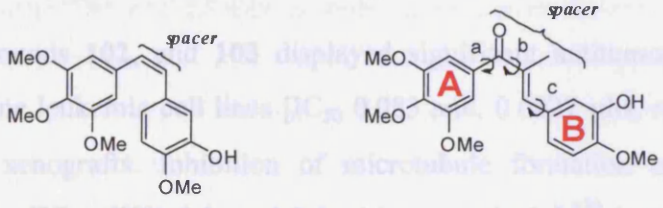
Chapter 6

6.0 Synthesis and evaluation of styrylchromones and quinazolines derivatives as cytotoxic and antitubulin agents

6.1 Introduction

Novel anticancer agents that target tumour vasculature as a consequence of their anti-tubulin properties are one of the subjects of a study described in this chapter. The importance of tubulin as a target has been highlighted by the discovery that the clinical candidate Combretastatin A4 (**15**) (Figure 40) displays potent and selective toxicity[326] towards tumor vasculature.[45] Our interest in tumor targeting compounds focuses on compounds related to **15**. A detailed structure-activity relationship (SAR) study conducted in our group led to the development of CA4-like chalcones[114] as inhibitors of tubulin polymerization. The α-methyl chalcone **47** inhibits cancer cell growth at low concentrations [IC_{50} (K562) 0.2 nM]. Chalcone **47** causes cell arrest at the G_2/M point and binds to the colchicine-binding site more strongly than colchicine itself. It also inhibits tubulin polymerization (IC_{50} 1.5 μM).

Figure 40. Structures of Combretastatin A4 (**15**) and chalcone **47**.



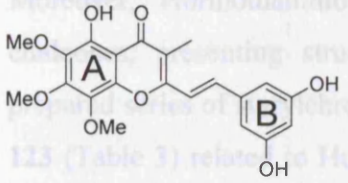
Combretastatin A4 (**15**)
IC_{50} (K562) 4.3 nM

47, *s-trans* conformer
IC_{50} (K562) 0.2 nM

It is believed that the spatial relationship between the two aromatic rings is an important feature that determines their ability to bind to tubulin.[100,101] The α,β-unsaturated carbonyl linker of chalcone **47** allows positioning of the aryl rings at an appropriate distance maximizing the interaction with the target. The X-ray crystal structure of **47** revealed that the carbon-oxygen and carbon-carbon double bonds are positioned *trans* relative to the C1-C2 single bond. Preliminary modeling and crystallographic studies led us to postulate that molecules adopting the *s-trans* conformation bind strongly to tubulin.[117]

The chemical aspect of the first part of the project is based on the styrylchromone natural product Hormothamnione (**101**) (Figure 41), isolated from the marine cryptophyte *Chrysophaeum taylori*.[327]

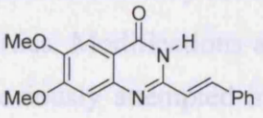
Figure 41. Structure of Hormothamnione (**101**).



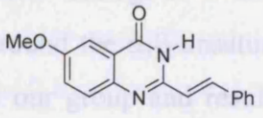
Hormothamnione (**101**)

Hormothamnione (**101**) is an exceptionally potent cytotoxin[327,328] and it has been shown to be a potent cytotoxin to several human leukemia cell lines.[327] While the mechanism of its cytotoxicity has not been fully characterized, it appears to operate via selective inhibition of RNA synthesis.[327] Synthesis, cytotoxicity, and inhibitory effect on tubulin polymerization of certain substituted styrylquinazolinones structurally related to Hormothamnione (**101**) have been previously reported in the literature (Figure 42).[329,330]

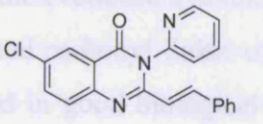
Figure 42. SAR and structures of styrylquinazolinones **102**, **103**, and **104**.



(**102**), IC_{50} (L1210) 0.083 μM, IC_{50} (ITP) 3.3 μM



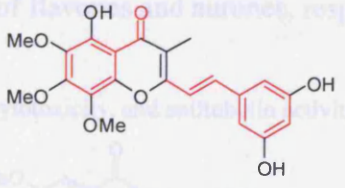
(**103**), IC_{50} (L1210) 0.0027 μM IC_{50} (ITP) 2.0 μM



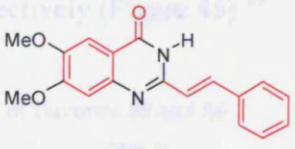
(**104**), IC_{50} (L1210) 0.64 μM, IC_{50} (K562) 1.05 μM, IC_{50} (ITP) 2.0 μM

In particular compounds **102**, and **103** displayed significant antitumour activity *in vitro* against L1210 murine leukemia cell lines [IC_{50} 0.083 and, 0.0027 μM, respectively] as well as humar tumour xenografts. Inhibition of microtubule formation appeared to be the mechanism of action [IC_{50} (ITP) 3.3, and 2.0 μM, respectively].[330] Interestingly, Jiang and coworkers report that compounds **102** and **103** showed only a weak effect on the colchicine binding.[330] However, to our best knowledge, no further studies elucidating the mechanism of action of this class of compounds have been reported in the literature to date. It is clear that styrylquinazolinones and Hormothamnione (**101**) share a similar skeleton except for different heteroatoms (Figure 43).

Figure 43. Hormothamnione (**101**) and quinazoline **102**. Common features are highlighted in red.



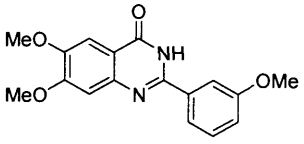
Hormothamnione (**101**)



(**102**)

Moreover, Hormothamnione (**101**) possesses remarkable structural similarity to the chalcones, presenting structural features for an effective interaction with tubulin. We prepared series of styrylchromones **109** (Table 1) and **113** (Table 2) and styrylquinazolinones **123** (Table 3) related to Hormothamnione (**101**) and initially assessed them for cytotoxicity against K562 human chronic myelogenous leukemia. Subsequent studies on the effect of the most potent compounds on tubulin polymerization were performed. We have synthesized styrylchromones and quinazolines in which the styryl aryl group has been introduced and varied to produce "unnatural" analogues of hormothamniome. Our choice of aryl groups includes groups that will improve the drug qualities of styrylchromones.

The second area of research focused on the synthesis of a new series of quinazolines **127** (Table 4) as conformationally-restricted analogs of **47** and the evaluated cytotoxicity and anti-tubulin properties. Modifications around the α,β-unsaturated carbonyl linker of chalcone **47** have been previously attempted in our group and resulted in good biological activity. The major goal of these modifications was to get an insight into the importance of the aryl ring orientation about the rotatable bond a and c in influencing the cytotoxicity and anti-tubulin properties (Figure 40). The anti-tubulin activity of numerous conformationally-restricted analogs of **47** has been amply investigated in our research group (e.g aurones,[125] flavones,[125,127]). Synthesis, citotoxicity, and inhibition of tubulin polymerization of various quinazoline derivatives have been previously described.[129,131-133] Compound **105**, despite its low cytoxicity in a panel of cancer cell lines, interestingly showed modest anti-tubulin properties [IC_{50} (ITP) 6.5 μM] (Figure 44).[131]

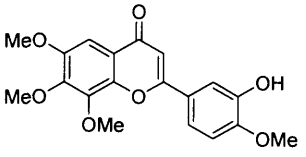


105, IC_{50} (ITP) 6.5 μM

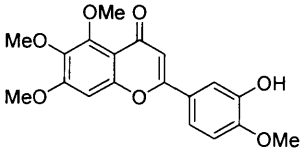
Figure 44. Structure of quinazolinone **105**.

The trimethoxybenzene moiety has featured in other antitubulin agents, and previous (SAR) revealed that the presence of the 6,7,8-trimethoxy and 5,6,7-trimethoxy A phenyl ring is optimum for relevant cytotoxicity and antitubulin activity of flavones and aurones, respectively (Figure 45).[84]

Figure 45. Structures, cytotoxicity, and antitubulin activity of flavones **55** and **56**.



54, IC_{50} (K562) 0.04 μM
IC_{50} (ITP) 25 μM



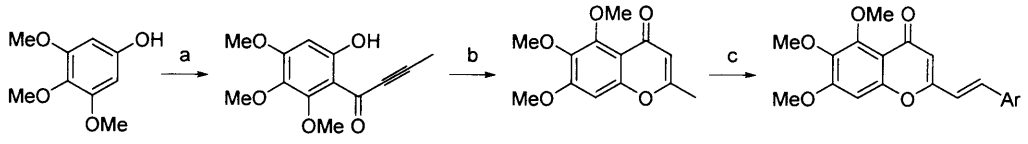
56, IC_{50} (K562) 22 μM
IC_{50} (ITP) > 50 μM

Based on these preliminary data, we designed and synthesized a focused library of 6,7,8-trimethoxy quinazolines **127**, conducting the SAR around the quinazoline *spacer* between the aryl rings and systematically investigating the substituent effect in the B ring.

6.2 Synthesis of styrylchromones, styrylquinazolines, and quinazolines derivatives

Styrylchromones **109** and **113** were initially synthesized in order to established the methoxy substitution pattern on the A ring favorable for optimal activity. The synthesis of styrylchromones **109** is depicted in Table 1. Treatment of 3,4,5-trimethoxyphenol (**106**) with an equimolar amount but-2-ynoic acid in Eaton's reagent (P_2O_5 in methansulfonic acid) afforded the ketone[331] intermediate **107**. ^{1}H NMR analysis of the crude reaction mixture revealed the formation of traces of chromone **108**. From a practical perspective, the intermediate **107** was not purified and the crude material was directly stirred in dry acetone in presence of K_2CO_3, under reflux for 2 h, to provide the cyclized chromone[331] **108** in overall 30% yield. Condensation of **108** with a series of substituted benzaldehydes furnished the desired library of styrylchromones[331] **109** for biological assessment (Table 1). Our experiments, carried out in basic media (NaOMe in MeOH), occasionally provided long reaction times (1 to 4 days), depending on the reactivity of the specific benzaldehydes employed. However, compounds of the series **109** crystallized from the reaction mixture and were generally isolated in good to moderate yield as single *trans* isomer, as observed by their ^{1}H NMR spectra.

Table 1. Synthesis of styrylchromones **109**.



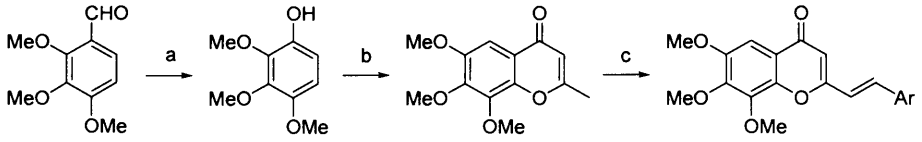
106 **107** **108**, 30% **109 a-k**

Key: a) P_2O_5, $MeSO_3H$, but-2ynoic acid, Ar, rt, 5 h; b) Acetone, K_2CO_3, Ar, 2 h, reflux; c) NaOMe/MeOH, ArCHO, 1-4 days, 80 °C.

Entry	Ar	Yield 108 → 109
109a	3,5-$(OMe)_2C_6H_3$	42%
109b	3,5-$(OBn)_2C_6H_3$	42%
109c	2,5-$(OMe)_2C_6H_3$	36%
109d	2,4,5-$(OMe)_2C_6H_2$	30%
109e	4-ClC_6H_4	44%
109f	3-ClC_6H_4	48%
109g	2-ClC_6H_4	43%
109h	3,4-$Cl_2C_6H_3$	45%
109i	2,4-$Cl_2C_6H_3$	41%
109j	2,6-$Cl_2C_6H_3$	42%
109k	4-$NO_2C_6H_4$	40%

The series of styrylchromones **113** isomeric to **109** was prepared as illustrated in Table 2. The synthesis relies upon the use of benzaldehydes as latent phenols. First 2,3,4-trimethoxybenzaldehyde (**110**) was oxidised in excellent yield (94%) to 2,3,4-trimethoxyphenol (**111**) by hydrogen peroxide in acidic methanol[332] (Table 2). Reaction of phenol **111** with but-2-ynoic acid in Eaton's reagent gave the chromone[331] **112** (Table 2). The formation of *o*-hydroxyarylethynyl ketone was not observed. The reaction was carried out at different temperatures (0 °C and room temperature) in order to investigate the product distribution and to improve the yield. In both cases only a poor yield (12%) of chromone **112** was isolated directly from the reaction mixture. However, this was sufficient for the preparation of a library of styrylchromones. Using the same conditions reported above for the synthesis of **109**, we prepared derivatives[331] **113** (Table 2).

Table 2. Synthesis of styrylchromones **113**.



110 **111**, 94% **112**, 12% **113 a-i**

Key: a) H_2O_2, H_2SO_4, MeOH, rt, 3 h; b) P_2O_5, $MeSO_3H$, but-2-ynoic acid, Ar, rt, 5h; c) NaOMe/MeOH, ArCHO, 1-4 days, 80 °C

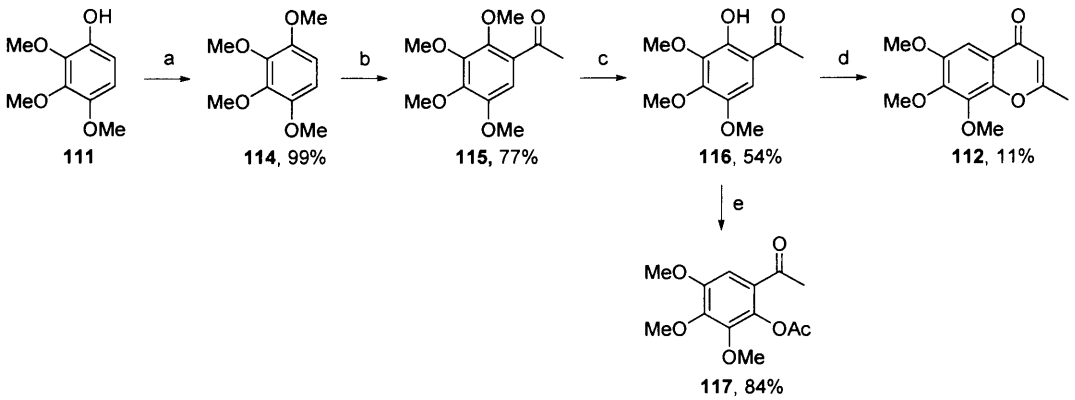
Entry	Ar	Yield 112 →113
113a	2-ClC_6H_4	59%
113b	3-ClC_6H_4	56%
113c	4-ClC_6H_4	58%
113d	2,4-$Cl_2C_6H_3$	63%
113e	2,6-$Cl_2C_6H_3$	65%
113f	3,4-$Cl_2C_6H_3$	68%
113g	3,5-$(OMe)_2C_6H_3$	37%
113h	2,5-$(OMe)_2C_6H_3$	34%
113i	2,4,5-$(OMe)_2C_6H_2$	37%

We also investigated an alternative route to the synthesis of the key intermediate **112** aiming at improving the yield and simplifying the tedious purification by chromatography on silica gel. This would also allow us to speed up the preparation of the target molecules expanding our compound collection. A base-assisted one-pot cyclization of various methoxy substituted 2-hydroxyacetophenone with easily available acylating reagents for the synthesis of chromones of type **112** has been previously described in the literature.[333] This protocol had been successfully followed in our group for the synthesis of the chromone scaffolds and involves the "DBU cyclization of 2-acetoxyacetophenones in pyridine. Unfortunately, the required starting material 2-hydroxy-3,4,5-trimethoxyacetophenone (**116**) is not

commercially available. However, we thought that it could be easily obtained from phenol **111** via sequential methylation , Friedel-Crafts acylation, and demethylation.

The phenol **111** was reacted with dimethyl sulfate in dry acetone in presence of excess potassium carbonate to give **114** in excellent yield[334] (99%) (Scheme 3). A literature reported[335] synthetic method [DCM, 0 °C, $AlCl_3$ (1.2 equiv) acyl chloride (1.1 equiv), 5 h] failed to provide the desired product **115** and the unreacted starting material was recovered. We investigated the effect of the temperature, carrying out the reaction at room temperature overnight. As shown by ^1H NMR spectra, a mixture of unreacted starting material and 2,3,4 trimethoxy phenol (**111**) was recovered.

Scheme 3. Synthesis of **112**.



Key: a) Acetone, K_2CO_3, Me_2SO_4, Ar, 22 h, reflux; b) $ZnCl_2$, Ac_2O, CH_3NO_2, Ar, 50 °C, 16 h; c) Benzene, $AlCl_3$, Ar, 5 h, 80 °C; d) DBU, Py, Ac_2O, 140 °C, Ar, overnight; e) DBU, Py, AcCl, 80 °C, Ar, overnight.

A comparable result and product distribution was noted when the reaction was performed at room temperature overnight using 3 equivalent of $AlCl_3$. We also attempted the reaction of **114** with 1 equivalent of $AlCl_3$ and acetyl chloride, in DCM, at 40 °C. The reaction was carried out until complete consumption of the starting material, as mesaured by TLC. Careful analysis of the ^1H NMR spectra of the crude material, revealed the formation of 2-hydroxyacetophenone **116**. Indeed, the *ortho*-phenolic proton of the 2-hydroxyacetophenone system (11.42 ppm) resonates at low field, due to the intramolecular hydrogen bond with the oxygen of the adjacent carbonyl group. Unfortunately this reaction was not regioselective and formation of demethylated side-products was also detected by ^1H NMR.

Finally, acetophenone **115** was obtained in a good yield (73%) reacting **114** in nitromethane, in presence of $ZnCl_2$, at 50 °C, under nitrogen and using acetic anhydride as acylating agent[336] (Scheme 3). Switching the solvent to acetonitrile afforded unreacted starting material. In conclusion, the solvent-Lewis acid system appeared to play a critical role in influencing

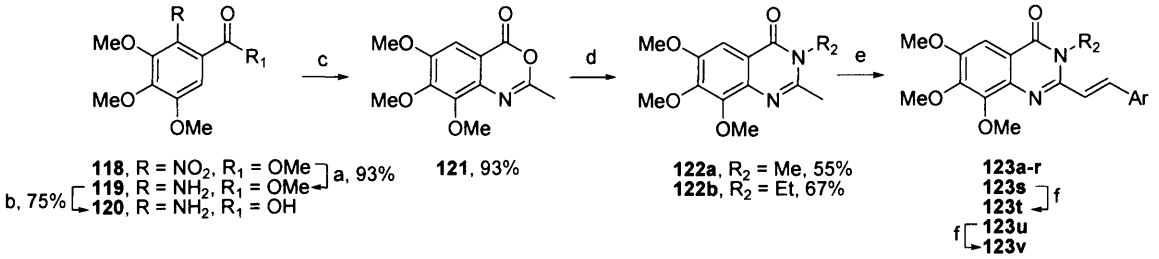
the reactivity of **114** towards the acylation over the demethylation and vice versa. In addition, more polar solvents are preferable. The demethylation,[335] carried out in benzene, in presence of $AlCl_3$, at 80 °C, for 16 h, gave compound **116** in moderate yield (Scheme 3).

Ketone **116** was reacted with acyl chloride and DBU in anhydrous pyridine as solvent, at 80 °C overnight. The cyclization did not occur, and ester **117** was recovered in 84% yield (Scheme 3). Ester **117** was obtained in comparable yield upon increasing the temperature to 140 °C. Chromone **112** was isolated in 11% yield when an equimolar amount of ketone **116** and acetic anhydride were reacted at 140 °C (Scheme 3). A longer reaction time (36 h), failed in providing a better yield.

Next, our strategy focused on identifying a chromone core replacement with improved potency. Mindful of the structural similarity shared by styrylchromones and styrylquinazolines (Figure 43), and of previous studies describing cytotoxicity and antitubulin properties of certain styrylquinazolines[329,330] (Figure 42), we directed our chemical strategy toward styrylquinazoline analogs **123** (Table 3). The quinazoline core would also provide easy access to the preparation of diverse sets of *N*-substituted derivatives through the synthesis of the key intermediate **122** (Table 3, step c).

Treatment of the methyl 2-nitro-3,4,5-trimethoxybenzoate (**118**) with tin(II) chloride in ethanol at 80 °C afforded the aminoester **119** (Table 3).[337] The key intermediate **121** was prepared by basic hydrolysis[338] of **119**, followed by cyclization[339] with acetic anhydride at 150 °C. The procedure for the synthesis of intermediates **122a** and **122b** is exemplified by the following reaction. Reaction of **121** with methylamine or ethylamine followed by cyclization in acidic media (glacial acetic acid and concentrated sulfuric acid) afforded compound **122a** and **122b**, respectively. Compounds **122a** and **122b** were then reacted with a series of substituted benzaldehydes to give the desired library **123** according to the procedure previously described for compounds **109** and **113**.

Table 3. Synthesis of styrylquinazolines **123**.

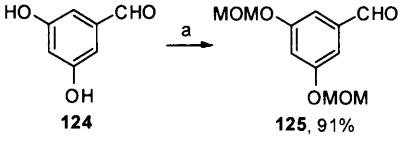


Key: a) $SnCl_2$, EtOH, 80 °C, 5 h; b) 50% aq. NaOH, 2-propanol, H_2O, 80 °C, 4 h; c) Ac_2O, 150 °C, 1h; d) THF, $MeNH_2$ or $EtNH_2$, rt, 20 min, ACOH, 100 °C, 1h; e) NaOMe/MeOH, ArCHO, 1-4 days, 80 °C; f) 6 M HCl, MeOH, reflux, 45 min.

Entry	R_2	Ar	Yield 122 →123
123a	Me	3 -ClC_6H_4	30%
123b	Me	4-ClC_6H_4	84%
123c	Me	2,4-$Cl_2C_6H_3$	58%
123d	Me	2,6-$Cl_2C_6H_3$	62%
123e	Me	3,4-$Cl_2C_6H_3$	31%
123f	Me	3,5-$(OMe)_2C_6H_3$	75%
123g	Me	2,5-$(OMe)_2C_6H_3$	57%
123h	Me	2,4,6-$(OMe)_2C_6H_2$	65%
123i	Me	2,4-$(OMe)_2C_6H_3$	56%
123j	Me	2,4,5-$(OMe)_2C_6H_2$	51%
123k	Me	3,4,5-$(OMe)_2C_6H_2$	38%
123l	Et	3,5-$(OMe)_2C_6H_3$	57%
123m	Et	2,5-$(OMe)_2C_6H_3$	64%
123n	Et	2,4-$(OMe)_2C_6H_3$	12%
123o	Et	2,4,5-$(OMe)_2C_6H_2$	49%
123p	Et	2,4,6-$(OMe)_2C_6H_2$	84%
123q	Et	3,4-$Cl_2C_6H_3$	20%
123r	Et	4-ClC_6H_4	57%
123s	Me	3,5-$(OMOM)_2C_6H_3$	34%
123t	Me	3,5-$(OH)_2C_6H_3$	90%
123u	Et	3,5-$(OMOM)_2C_6H_3$	31%
123v	Et	3,5-$(OH)_2C_6H_3$	95%

The MOM-protected benzaldehyde (**125**) employed for the synthesis of **123a** and **123w** is not commercially available and it was prepared by reacting 3,5-dihydroxybenzaldehyde (**124**) with MOM-chloride in DMF in presence of sodium hydride (Scheme 4).

Scheme 4. Synthesis of building block **125**.

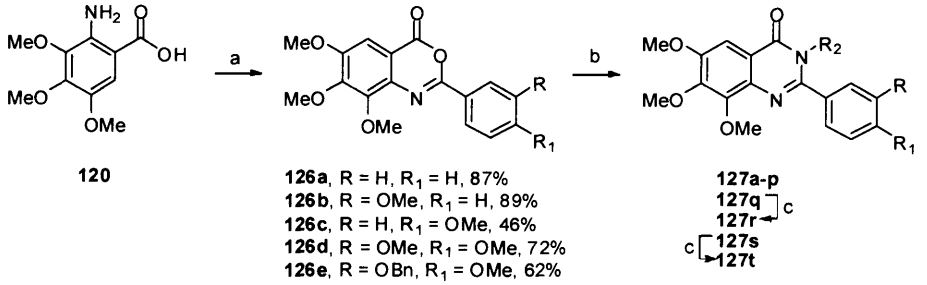


Key: a) NaH, DMF, Ar, 0 °C, 30 min, MOM-chloride, rt, 1h.

Moreover, compounds of the series **127** crystallized from the reaction mixture and were generally isolated in good to moderate yield as single *trans* isomer, as determined from their ^1H NMR spectra. The MOM group was cleaved under mild acid condition to afford the target compounds **123o** and **123z** in excellent yields.[340]

Finally, 6,7,8-trimethoxy quinazolines **127** (Table 4) were synthesized as conformationally-restricted analogs of **47**. SAR was conducted around the quinazoline *spacer* between the aryl rings and systematically investigating the substituent effect in the B ring. The effects of the substitution on the quinazolinone N atom was also investigated. N-methyl (**127e-h**) derivatives were prepared as direct comparison to **47**. To study the degree of steric bulk that could be tolerated *N*-ethyl (**127i-l**) and *N*-propyl (**127m-p**) were also prepared.
Quinazolines **127** were synthesized according to the procedure reported in Table 4.

Table 4. Synthesis of quinazolines **127**.



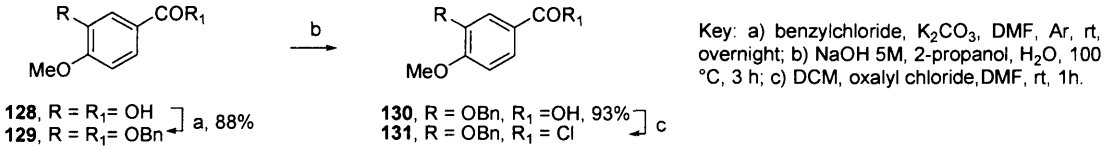
Key: a) Substituted benzoyl chloride, pyridine, rt, 1 h; b) RNH_2, pyridine, μw, 150 °C, 30 min; c) H_2, 10% Pd/C, THF, MeOH, rt, 3 h.

Entry	R	R_1	R_2	Yield 126 → 127
127a	H	H	H	55%
127b	OMe	H	H	68%
127c	H	OMe	H	69%
127d	OMe	OMe	H	57%
127e	H	H	Me	47%
127f	OMe	H	Me	67%
127g	H	OMe	Me	21%
127h	OMe	OMe	Me	56%
127i	H	H	Et	13%
127j	OMe	H	Et	19%
127k	H	OMe	Et	6%
127l	OMe	OMe	Et	28%
127m	H	H	Pr	4%
127n	OMe	H	Pr	34%
127o	H	OMe	Pr	8%
127p	OMe	OMe	Pr	14%
127q	OBn	OMe	H	68%
127r	OH	OMe	H	95%
127s	OBn	OMe	Me	74%
127t	OH	OMe	Me	92%

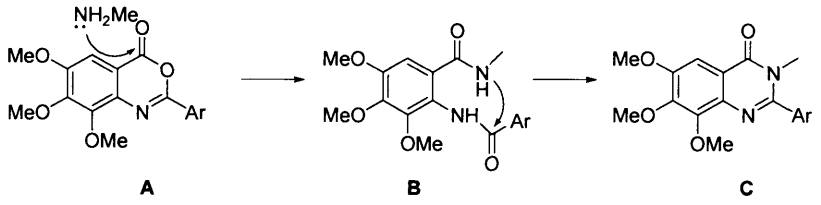
The 3,4,5-trimethoxyanthranilic acid (**120**) was reacted with a range of benzoyl chlorides to afford the desired intermediates **126**.[337] The benzyl-protected benzoyl chloride **131** was prepared by reacting 3-hydroxy-4-methoxybenzoic acid (**128**) with benzyl chloride to give ester **129**. Saponification of **129**, followed by chlorination afforded the intermediate **131**, which was used in the next step without further purification (Scheme 5). The synthesis of

library **127** began with intermediate **126a**, following the procedure reported for the synthesis of **122a** and **122b**. Complete consumption of the starting material was observed by TLC after the addition of methyl amine (aq, 40%). Heating the reaction mixture in glacial acetic acid in presence of concd H_2SO_4 failed in affording quinazoline **127e**. As viewed by ^1H NMR of the crude material, an unknown compound was recovered, whose structure was thought to be **B** (Scheme 6). As shown in Scheme 5, the mechanism of the quinazoline formation starts giving the intermediate **B**. Intramolecular nucleophilic attack followed by ring closure gives quinazoline **C**. The intermediate **B** deriving from the reaction of **126a** with methylamine appeared to be very stable under the conditions reported above. Therefore, harsher conditions were thought to be necessary to push the reaction toward the formation of quinazole **127e**.

Scheme 5. Synthesis of building block **131**.



Scheme 6. Mechanism and intermediate in the convertion of **126** to **127**.



Higher temperature was thought to be the possible key of the success of the reaction. Pyridine was chosen as suitable medium to perform the reaction. We also believed that the reaction could be effected by microwave heating. Compounds **127** were synthesized according to the conditions reported in Table 4 and obtained in moderate yields for the *N*-H, *N*-methyl and derivatives. Significantly lower yield were observed for the *N*-ethyl and *N*-propyl analogues. Starting material was never recovered and or detected by TLC analysis or NMR spectroscopy. The reduced reactivity of the *N*-ethyl and *N*-propyl analogues may be due to the enhanced steric hindrance of the substrate. Moreover, only traces of product formation were detected when the synthesis of the *N*-isopropyl analogue was attempted (data not shown). Finally, deprotection of compounds **127s** and **127u** was carried out under an atmospheric pressure of hydrogen to afford the corresponding hydroxyl compounds **127t** and **127v** in excellent yields (Table 4).

6.3 Biological results and discussion

All the synthesized compounds were tested in a preliminary MTT Cell Proliferation Assay in the K592 cell line as described by Edmondson *et al.*[341] The MTT assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The reduction of the yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is a reliable way to examine cell proliferation. The tetrazolium salts is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes to generate the resulting intracellular blue-formazan derivative (Scheme 7) which can be solubilized and the concentration determined by optical density at 570 nm. The IC_{50} value represents the concentration which results in a 50% inhibition in cell growth after 5 days incubation. Combretastatin A4 (**15**) and chalcone **47** (also referred to as SD400) were used as positive controls.

Scheme 7. Primary reaction in the MTT assay.

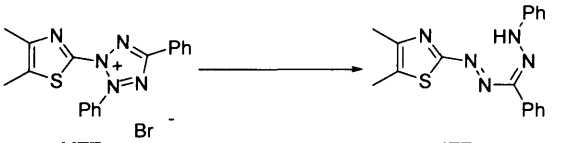


Table 5 summarizes the cytotoxicity assay of styrylchromones **109**, **113** and quinazoline **123**. We initially evaluated the "methoxy substitution effect" on the A ring. As shown for the compounds series **109** and **113**, we found that the 5-OMe dramatically decreased the activity. In contrast, the 6,7,8-trimethoxy A-ring arrangement exhibited the greatest cytotoxicity (e.g. compare **109c**, **109f**, to**113b**, and **113h**), resembling the same cytotoxicity trend observed for structurally related to aurones,[125] and flavones[125] and therefore indicating potentially a common mode of action.

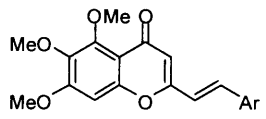
The cell growth inhibition data of our library **113** indicated that 2,5-dimethoxy substitution on the B ring (**113h**, IC_{50} 79.9 nM) gave rise to the most potent compound. Switching the methoxy group to the 3-position (**113g**) caused a significant decrease in cytotoxicity (approximately 5-fold), while introduction of an additional 4-OMe group resulted in loss of potency (**113i**, IC_{50} > 10 μM). Moderate cytotoxicity was also observed in the case of compounds **113a** and **113b**, bearing a chlorine group at the 2- and 3-position, respectively. The 4-Cl substitution resulted detrimental for good activity (**113c**). Furthermore, compounds **113d**, **113e** and **113f**, with dichlorobenzene ring, exhibited drastically reduced cytotoxicity.

Having established the preliminary SAR of the B ring in the series **109** and **113**, the 6,7,8-trimethoxyquinazoline moiety was examined as an alternative to the chromone scaffold and found to be less potent. To our delight, the cytotoxicity profile of series **123** well correlates to series **109** and **113**, possibly indicating that a common mode of action is operating. As typical results, compounds **123g** and **123m** are about 7-fold less cytotoxic than the corresponding chromone derivative **113h**. Generally, substitution of R with methyl or ethyl group does not affect the activity.

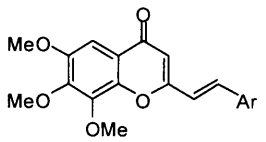
Table 5. Cell growth inhibition against[a] the K592 cell line.

Entry	R	Ar	IC_{50} (μM)
[a]**109a**	-	3,5-$(OMe)_2C_6H_3$	>10
[a]**109b**	-	3,5-$(OBn)_2C_6H_3$	>10
[a]**109c**	-	2,5-$(OMe)_2C_6H_3$	>10
[a]**109d**	-	2,4,5-$(OMe)_2C_6H_2$	>10
[a]**109e**	-	4-ClC_6H_4	>10
[a]**109f**	-	3-ClC_6H_4	>10
[a]**109g**	-	2-ClC_6H_4	>10
[a]**109h**	-	3,4-$Cl_2C_6H_3$	>10
[a]**109i**	-	2,4-$Cl_2C_6H_3$	>10
[a]**109j**	-	2,6-$Cl_2C_6H_3$	>10
[a]**109k**	-	4-$NO_2C_6H_4$, 40%	>10
[a]**113a**	-	2-ClC_6H_4	1
[a]**113b**	-	3 -ClC_6H_4	2.9
[a]**113c**	-	4-ClC_6H_4	>10
[a]**113d**	-	2,4-$Cl_2C_6H_3$	>10
[a]**113e**	-	2,6-$Cl_2C_6H_3$	>10
[a]**113f**	-	3,4-$Cl_2C_6H_3$	>10
[a]**113g**	-	3,5-$(OMe)_2C_6H_3$	0.39
[a]**113h**	-	2,5-$(OMe)_2C_6H_3$	79.9 nM
[a]**113i**	-	2,4,5-$(OMe)_2C_6H_2$	>10
[a]**123a**	Me	3 -ClC_6H_4	9.3
[a]**123b**	Me	4-ClC_6H_4	>10
[b]**123c**	Me	2,4-$Cl_2C_6H_3$	>10
[a]**123d**	Me	2,6-$Cl_2C_6H_3$	>10
[b]**123e**	Me	3,4-$Cl_2C_6H_3$	>10
[b]**123f**	Me	3,5-$(OMe)_2C_6H_3$	1.4
[b]**123g**	Me	2,5-$(OMe)_2C_6H_3$	0.59
[b]**123h**	Me	2,4,6-$(OMe)_2C_6H_2$	>10
[b]**123i**	Me	2,4-$(OMe)_2C_6H_3$	8.5
[b]**123j**	Me	2,4,5-$(OMe)_2C_6H_2$	>10
[b]**123k**	Me	3,4,5-$(OMe)_2C_6H_2$	>10
[c]**123t**	Me	3,5-$(OH)_2C_6H_3$	2
[d]**123l**	Et	3,5-$(OMe)_2C_6H_3$	4.49
[d]**123m**	Et	2,5-$(OMe)_2C_6H_3$	0.45
[d]**123n**	Et	2,4-$(OMe)_2C_6H_3$	4.26
[d]**123o**	Et	2,4,5-$(OMe)_2C_6H_2$	>10
[d]**123p**	Et	2,4,6-$(OMe)_2C_6H_2$	>10
[d]**123q**	Et	3,4-$Cl_2C_6H_3$	>10
[d]**123r**	Et	4-ClC_6H_4	>10
[c]**123v**	Et	3,5-$(OH)_2C_6H_3$	1.8

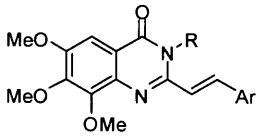
Key: a) CA4 IC_{50} 5.6 nM; b) CA4 IC_{50} 6.4 nM; c) CA4 IC_{50} 1.54 nM; d) CA4 IC_{50} 1.19 nM.



109



113



123, R = Me, Et

A similar trend was observed in the subtitution pattern on the B ring of series **123**. The 2,5-dimethoxy substitutions in the B ring are critical for cytotoxicity (**123g** and **123m**, IC_{50} 0.59 and 0.45 μM), while the 3,5-dimethoxy substitution results in drop in potency (**123f** and **123l** IC_{50} 1.4 and 4.49 μM). Substitutions at the 4-position in the B ring are not tolerated or compromise the activity as shown by compounds **123b**, **123i**, **123t**, and **123n**. Trimethoxy substitution of B ring provides a reduction of activity as illustrated by comparison of compounds, **123h** and **123j**, **123k** and **123o** and **123p**. Replacement of methoxy groups with chlorine generally results in a significant drop in potency (compounds **123a-e**, and **123q,r**)

The increased cytotoxicity of compounds of series **113**, and **123** bearing methoxy substituent suggests that electron-donating groups might be favorable for cytotoxicity and a probable engagement of the oxygen as hydrogen bond acceptor. Moreover, the hydroxyl group appears to be well tolerated: compounds **123t** and **123v** exhibited a potency in the micromolar range comparable to that of the corresponding methylated analogues **123f** and **123l**. It would appear that the hydroxyl group does not act as a hydrogen bond donor.

Table 6 summarizes the cytotoxicity assay of compounds of series **127**. Among the new compounds, **127c** and **127r** resulted as the only active ones (IC_{50} 9.3 and 8.1 μM, respectively). Quinazoline **127r** is significantly less active than chalcone **47** and the corresponding flavones **55** and **132**[125](Figure 46), providing further evidence that the conformational restriction of **47** about bond a and c results in lower cytotoxicity, and that the replacement of the α,β-unsaturated moiety with the isosteric quinazoline ring to position the two aryl rings is not tolerated.

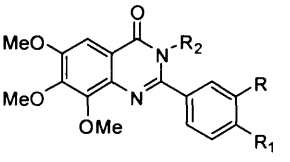
When R_2 is substituted with methyl, ethyl or propyl group a drop in activity is observed as shown by compounds **127g**, **127k**, **127o**, **127t**. If this is due to an enhanced tubulin binding activity of derivative **127c** and **127t**, the NH may be involved in a hydrogen bond interactions acting as hydrogen donor. Steric effects can also reduce the activity of these compounds.

It has been previously reported[84] that the presence *para*-methoxy group in the B ring is critical for the good activity of CA4 analogues. In accordance, derivatives **127a**, **127b**, exhibited a decreased cytotoxicity. The detrimental role of the *meta*-methoxy group is also revealed by the loss of activity of compound **127d**. The *meta* OMe-OH substitution proved to be successful for giving a compound (**127t**) of dramatically improved cytotoxicity. In agreement with Cushman's seminal work,[84] the comparable potency of compounds **127c** and **127t** highlights that the presence of the hydroxy group is important but not necessary for potency.

Table 6. Cell growth inhibition against[a] the K592 cell line.

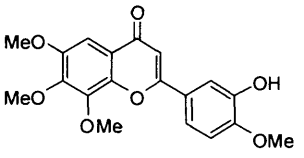
Entry	R	R1	R2	IC_{50} (μM)
127a	H	H	H	>10
127b	OMe	H	H	>10
127c	H	OMe	H	9.3
127d	OMe	OMe	H	>10
127e	H	H	Me	>10
127f	OMe	H	Me	>10
127g	H	OMe	Me	>10
127h	OMe	OMe	Me	>10
127i	H	H	Et	>10
127j	OMe	H	Et	>10
127k	H	OMe	Et	>10
127l	OMe	OMe	Et	>10
127m	H	H	Pr	>10
127n	OMe	H	Pr	>10
127o	H	OMe	Pr	>10
127p	OMe	OMe	Pr	>10
127q	OBn	OMe	H	>10
127r	OH	OMe	H	8.1
127s	OBn	OMe	Me	>10
127t	OH	OMe	Me	>10

Key: a) MTT assay, Cell Line K562, CA4 IC_{50} 2.2 nM, SD400 IC_{50} 2.7nM

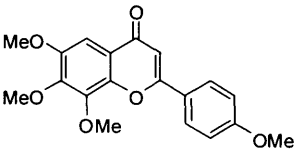


127

Figure 46. Structures and in vitro SAR of flavones **55** and **132**.



55, IC_{50} (K562) 0.04 μM



132, IC_{50} (K562) 0.83 μM

Among the synthesized compounds we selected those analogues showing significant cytotoxicity (generally defined as IC_{50} value < 1.5 μM), and evaluated for activity *in vitro* tubulin polymerization inhibition assay. Samples were prepared directly in quartz cuvettes at 0 °C and contained Mes buffer [(2-(morpholino)ethanesulfonic acid), EGTA (ethyleneglycol-*bis*-(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid), $MgCl_2$, distilled water, pH 6.6)], GTP (Guanosine 5'-triphosphate), tubulin, and the candidate drug (in DMSO). The tubulin/drug samples were immediately placed in a Varian Cary 300 Bio UV/visible spectrophotometer, preheated at 37 °C, alongside six blank samples containing Mes buffer and GTP. Recording the absorbance (λ 350 nm) for a period of 20 minutes, the results were compared to the untreated control cells to evaluate the relative degree of change in optical density.

All the compounds were tested at one concentration (10 μM) and compared the % of tubulin assembly with CA4 at the same concentration (Table 7).

Table 7. Effect upon tubulin binding for compounds of series **113** and **123**.

Entry	% tubulin assembly	% inhibition
113a	67	33
113b	110	-10
113g	81	19
113h	62	38
123f	72	28
123g	82	18
123m	84	16
CA4	14	86
DMSO	100	0

The lower antitubulin activity of the selected compounds correlated well with their reduced cytotoxic potency with respect to CA4. Although compound **113h** determines cell growth inhibition in K562 cell line, its IC_{50} is about 14 times higher than that of reference compound CA4 (**15**) (Table 5). Compound **113h** may exert its inhibitory effect on cell growth through an interaction with different targets. Interestingly, compound **113b** seems to promote tubulin assembly. However, further experiments are needed to determine whether its cytotoxocity is originated from an alternative mode of action. A detailed SAR and a detailed characterization of these compounds with tubulin need to be conducted in order to enhanced the cytotoxic activity and antitubulin properties of the styrylchromone and styrylquinazolinone derivatives and elucidate the mode of action.

6.4 Conclusion

Cytotox styrylchromones **109**, and **113**, and styrylquinazolinoes **123**, related to Hormothamnione (**101**) and chalcone **47** have been investigated. Despite their lower potency compared to the initial lead **47**, the cytotoxicity of these compounds appeared to be dependent on the substitution on the chromone and quinazolinone scaffold indicating that the 6,7,8-trimethoxy substitution is good for activity. The 2,5-dimethoxy substitution at the styryl-aryl terminus appeared to be critical for good cytotoxicity.

A new series of quinazolinones **127** was also prepared as conformationally-restricted analogs of **47** and the evaluated cytotoxicity and anti-tubulin properties. Quinazoline **127t** is significantly less active than chalcone **47** and the corresponding flavones **55** and **132**, providing further evidence that the conformational restriction of **47** about bond a and c results in lower cytotoxicity.

Chapter 7

7.0 Design and synthesis of potential inhibitors of STAT3 dimerization

7.1 Introduction

In the effort to discover novel potential inhibitors of STAT3 dimerization in a virtual screening mode, the NCI compound collection was docked into the pTyr-binding pocket of STAT3 SH2 domain, derived from the X-ray crystal structure of pSTAT3 bound to a fragment of DNA (TGCATTTCCCGTAAATCT) (pdb code IBG1).[154] The observed docking score relative to the native phosphopeptide sequence APY*LK was –11.9 Kcal/mol. Docking of the native peptide also indicated a T-shape binding model (Figure 47).

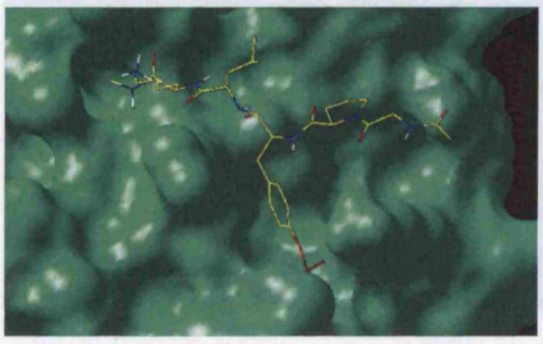
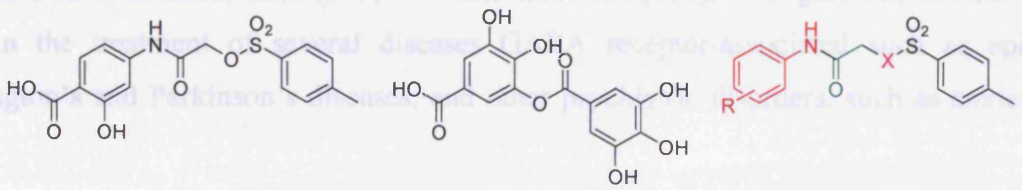


Figure 47. The peptide fragment APY*LK bound to the pTyr-binding pocket of STAT3.

Relying upon computational modeling of the native peptide, computational analyses identified two compounds **NSC64859** and **NSC59263** (Figure 48), which inhibit STAT3 activity *in vitro* with IC_{50} values of 96 and 72 μM, respectively. Both compounds possess a benzoic acid group, which is capable of acting as a good phosphotyrosine mimic. The design of the first generation library was based on **NSC64859**, since it is arguable more drug-like. The synthesis of **NSC59263** analogues will require significant protecting group manipulation to control, the reactivity of the different hydroxyl groups.

Figure 48. The initial hits **NSC64859** and **NSC59263** identified from the virtual screening of the NCI compound collection. The four points of diversity of the **NSC64859** scaffold.



NSC64859
IC_{50} 96 μM, Docking score -9.8

NSC59263
IC_{50} 72 μM, Docking score -9.7

Scaffold of **NSC64859** analogs

The general scaffold of **NSC64859** has four points of diversity: the carbonyl linker, the X linker, the arylsulfonylgroup and the arylamine moiety (Figure 48). In these compounds it is

possible to vary the X linker by replacing X with O, N or CH_2. Different amides can be prepared by reacting with commercially available anilines, that were selected to have phosphatase mimicking group. The proposed structures were docked in a virtual screen and only the compounds with the higher docking scores were prepared. Docking of the first library suggested two different modes of binding of the library members with the arylsulfonylgroup binding in either pockets A or B (Figure 49).

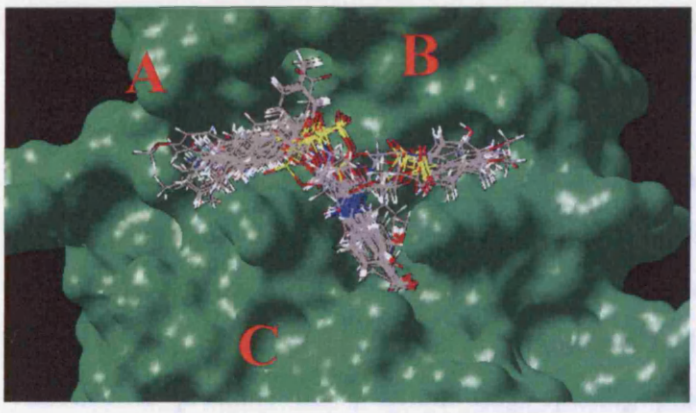


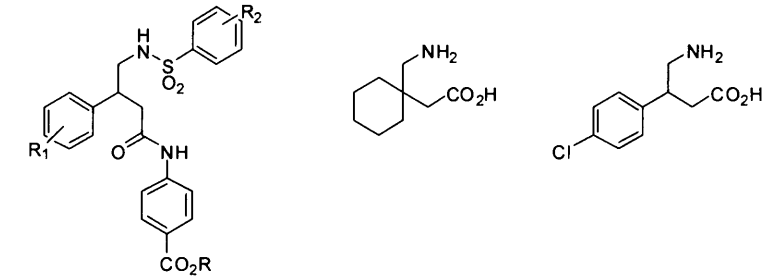
Figure 49. Cluster overlay of all library members docked to pTyr binding site.

The docking studies led us to the development of the T-shape model of molecules that can occupy both pockets A and B, thereby increasing their binding affinity (Figure 49). This design of the virtual second-generation library is also supported by the binding mode of the native peptide, although the site B is not occupied by the peptide. The structures were docked and the top docking compounds were selected for synthesis.

7.2 The synthesis of the T-shape model of molecules *via* conjugate addition of nitromethane

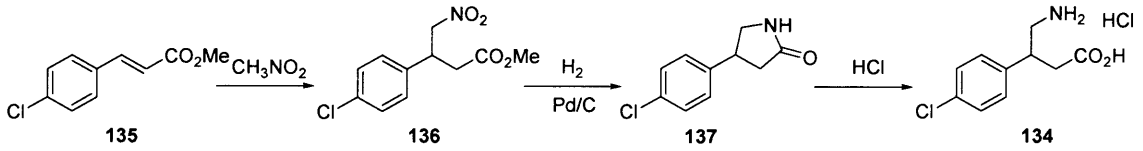
The T-shape model incorporates some of the features of GABA (γ–amino butyricacid) analogues such as Gabapentin (**133**)[342,343] and Baclofen (**134**),[344] (Figure 50) clinical agents used in the treatment of several diseases GABA receptor-associated such as epilepsy, Huntington's and Parkinson's diseases, and other psychiatric disorders, such as anxiety and pain.

Figure 50. Sharing features of the T-shape scaffold and the GABA analogues.



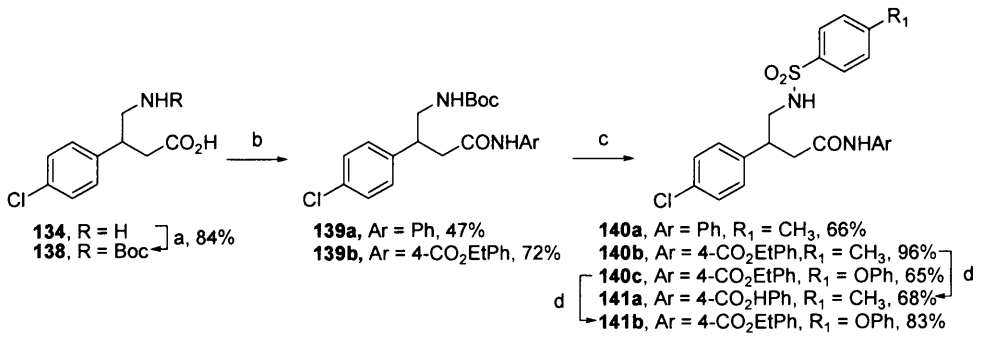
Due to its pharmacological activity, many methods have been developed for the synthesis of GABA-analogue Baclofen (**134**).[345-359] One of the most attractive methods (Scheme 1) for the construction of Baclofen (**134**) involves the conjugate addition of nitromethane to the methyl 4-chlorocinnamate (**135**) (Scheme 8).[359-363] The resulting γ-nitroester **136** can be easily converted into the corresponding γ-lactam **137** by reduction of the nitro group under an atmospheric pressure of hydrogen.[359-361] Aqueous hydrochloric acid has been found to be effective to promote the γ-lactam ring opening producing the desired product **134** as corresponding hydrochloride salt.[364]

Scheme 8. Synthetic route to Baclofen (**134**).



We envisioned that the T-shape molecules derived from **134** may be rapidly accessed *via* Boc-anhydride protection → amide coupling → deprotection → amide coupling synthetic sequence (Scheme 9). Compounds **140a**, **140b**, **140c**, **141a** and **141b** were prepared from the commercially available (±) Baclofen (**134**) according to the route depicted in Scheme 9.

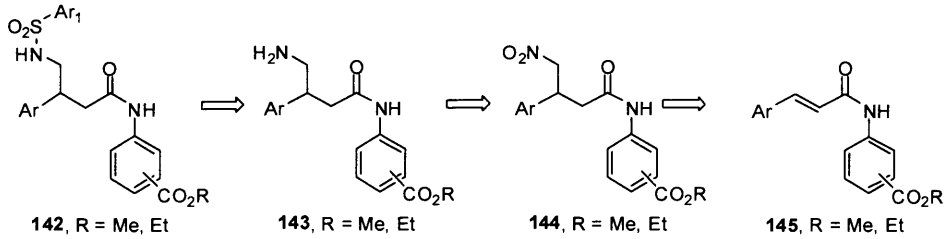
Scheme 9. Synthetic route to target compounds **140** and **141**.



Key: a) Boc_2O, 1.4-dioxane, H_2O, NaOH 1M, rt, 4 h; b) DMF, HATU, DIPEA, aniline or ethyl 4-aminobenzoate, rt, Ar, overnight; c) DCM, TFA, rt, 2 h, 4-phenoxybenzenesulfonyl chloride or tosyl chloride, K_2CO_3, dioxane, H_2O, rt, 2-3 h; d THF, MeOH, NaOH 1M, rt, overnight.

Although established, this approach did not meet our need of a general and straightforward synthetic route which would allow us to prepare the target T-shape molecules in a combinatorial fashion. Retrosynthetic analysis revealed there may be an alternative method of constructing the T-shape scaffold. This approach, which centered on the generation of nitro derivative **144** as the key step for the preparation of **142** (Scheme 10), was particularly attractive. This synthetic strategy relied upon a Michael reaction of nitromethane with **145**, easily accessible by reacting acid chlorides with the appropriate anilines (Scheme 10).

Scheme 10. Alternative retrosynthetic approach to the synthesis of the scaffold of T-shape molecules.

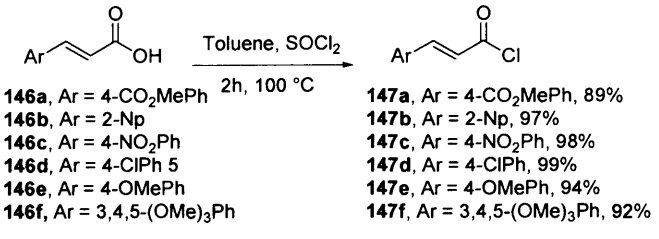


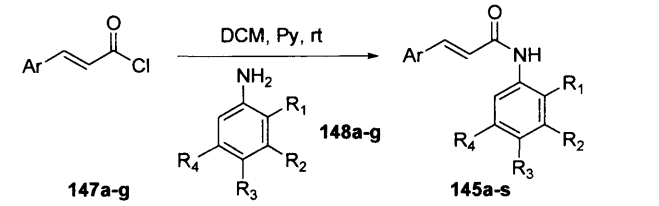
To our knowledge, α,β-unsaturated esters,[354,360-369] and nitroolefins[370-374] had been typically employed as substrates in the conjugate addition of nitromethane, while no example has been reported on the Michael addition of nitromethane to amides, probably due to the fact that they are less reactive than α,β-unsaturated esters and nitroolefins.

We envisioned that a better reactivity of the electron-poor amides **145** toward the conjugate addition might derive from the activation of an electron-withdrawing carbonyl group on the phenyl ring at the amide-terminus (Scheme 10). Following this hypothesis, we undertook the investigation of the conjugate addition of nitromethane to amides **145** and developed a versatile and efficient methodology. As a preliminary study, the substrates of choice were

derivatives **145b**, **145c**, and **145r**. Table 8 outlines the conditions followed for the synthesis of the amides **145**. The two-step process involved first the conversion of the acid into the acid chloride using thionyl chloride,[375] followed by the coupling itself with the appropriate anilines[376-378] **148a-g**. Various substituted cinnamic acids were commercially (**146c-f**) available or easily prepared (**146a,b**) from the corresponding aromatic aldehydes by Knoevenagel condensation using malonic acid.[379]

Table 8. Synthesis of acid chlorides **147** and amides **145**.





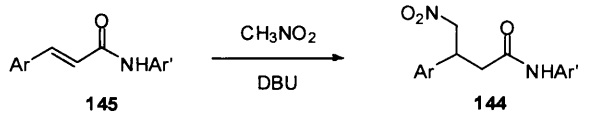
Substrate	Substrate	Ar	R_1	R_2	R_3	R_4	Product	Yield
147a	**148a**	4-CO_2MePh	CO_2Me	H	H	H	**145a**	84%
147b	**148b**	2-Np	H	H	CO_2Et	H	**145b**	80%
147b	**148c**	2-Np	H	OH	CO_2Me	H	**145c**	79%
147c	**148a**	4-NO_2Ph	CO_2Me	H	H	H	**145d**	72%
147c	**148d**	4-NO_2Ph	H	CO_2Me	H	H	**145e**	80%
147c	**148e**	4-NO_2Ph	H	H	CO_2Me	H	**145f**	84%
147d	**148a**	4-ClPh	CO_2Me	H	H	H	**145g**	84%
147d	**148d**	4-ClPh	H	CO_2Me	H	H	**145h**	40%
147d	**148e**	4-ClPh	H	H	CO_2Me	H	**145i**	41%
147e	**148a**	4-OMePh	CO_2Me	H	H	H	**145j**	86%
147e	**148d**	4-OMePh	H	CO_2Me	H	H	**145k**	60%
147e	**148e**	4-OMePh	H	H	CO_2Me	H	**145l**	72%
147f	**148a**	3,4,5-(OMe)$_3$Ph	CO_2Me	H	H	H	**145m**	96%
147g	**148a**	Ph	CO_2Me	H	H	H	**145n**	84%
147g	**148d**	Ph	H	CO_2Me	H	H	**145o**	66%
147g	**148e**	Ph	H	H	CO_2Me	H	**145p**	88%
147g	**148f**	Ph	H	H	H	H	**145q**	98%
147g[a]	**148b**	Ph	H	H	CO_2Et	H	**145r**	-
147a	**148g**	4-CO_2MePh	NO_2	H	H	H	**145s**	93%

a) The crude material was used in the next step without further purification.

An examination of several conditions reported in the literature for the addition of nitromethane to α,β-unsaturated carbonyls, revealed the "nitromethane-DBU" couple as the most suitable solvent-base system to investigate the reactivity of our substrates. Our initial experiments, carried out employing 1.1 equiv of DBU at room temperature, provided long

reaction times (Table 9, entries 1, 2, and 3). To investigate the effect of the temperature, a series of experiments were performed in the microwave reactor under different conditions. When compound **145r** was subjected to the reaction with nitromethane, after 15 min at 60 °C, 33% conversion was observed by ^{1}H NMR (Table 9, entries 4). The conversion could be dramatically increased when the reaction was conducted at 100 °C, with an improvement in the yield (Table 9, entries 5). The optimized conditions could be successfully applied to compounds **145b** and **145c** (Table 9, entry 6 and 7).

Table 9. Condition optimization of nitromethane addition to **144**.



145 **144**

Entry[a]	Substrate	T (°C)	Reaction Time	Product	% Conversion[c]	Yield
1	**145r**	rt	2 days	**144a**	81%	57%
2	**145b**	rt	3 days	**144b**	95%	46%
3	**145c**	rt	3 days	**144c**	100%	55%
4[b]	**145r**	60 °C	15 min	**144a**	33%	-
5[b]	**145r**	100 °C	15 min	**144a**	100%	68%
6[b]	**145b**	100 °C	15 min	**144a**	100%	70%
7[b]	**145c**	100 °C	15 min	**144a**	100%	55%
8[b]	**145q**	rt	4 days	**144d**	32%	-
9[b]	**145q**	60 °C	15 min	**144d**	16%	-
10[b]	**145q**	100 °C	15 min	**144d**	63%	-
11[b]	**145q**	100 °C	30 min	**144d**	82%	-
12[b]	**145q**	150 °C	15 min	**144d**	100%	50%

a) All the experiments were carried out employing 1.1 equivalent of DBU. b) The experiments were carried out in the Biotage microwave reactor. c) % Conversion was determined by ^{1}H NMR spectroscopy.

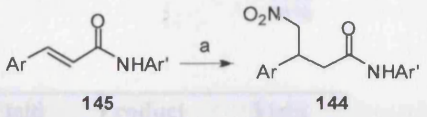
The effect of the electron-withdrawing group on the reactivity of model compounds **145a**, **145b**, and **145r** was examined. As expected, when 3,*N*-diphenyl-acrylamide (**145q**) was reacted with nitromethane at room temperature, after 4 days a very low conversion was observed by ^{1}H NMR (Table 9, entry 8). Only 63% conversion could be noted under the optimized conditions (Table 9, entry 10). However, the complete conversion of **144q** into **144d** was proved to be achievable upon increasing the reaction time (Table 9, entry 11), or at elevated temperature (Table 9, entry 12).

A variety of amides were investigated under the optimized conditions, as summarized in Table 10. The reactivity of *N*-phenyl substituted amides bearing the methyl ester group at the *ortho*, *meta*, and *para* positions was examined. A broad range of electron-withdrawing and electron-donating groups on the cinnamic acid-terminus of the amides, were well tolerated and good yields were observed in all the cases. Structures of compounds **144e-n** were

confirmed by their spectroscopic data. In addition, structural confirmation of **144e** (Figure 51A) and **144l** (Figure 51B) was carried out by single-crystal X-ray diffraction.

Table 10. Microwave-assisted nitromethane addition to **144**.

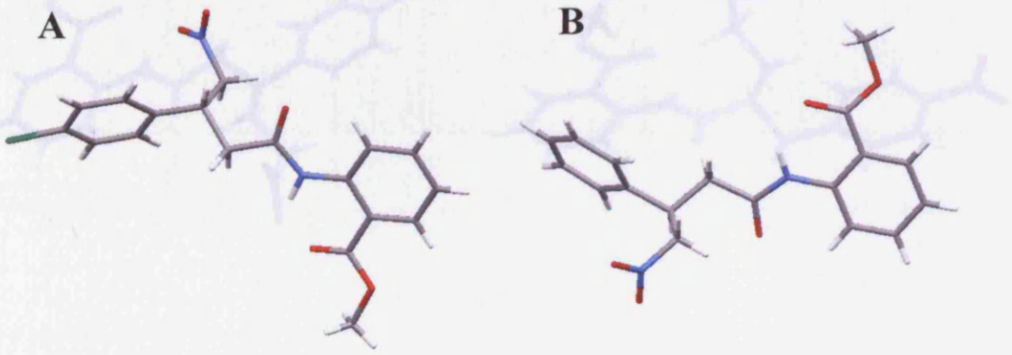
Entry	Substrate	Product	Yield
1	**145g**	**144e**	35%
2	**145h**	**144f**	77 %
3	**145i**	**144g**	71%
4	**145j**	**144h**	40 %
5	**144k**	**144i**	69%
6	**145l**	**144j**	57%
7	**145m**	**144k**	34%
8	**145n**	**144l**	51%
9	**145o**	**144m**	70%
10	**145p**	**144n**	81%



145 **144**

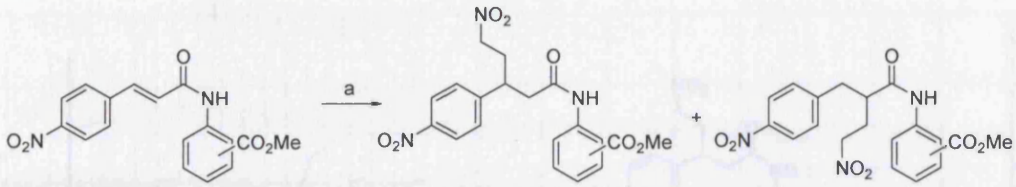
Key: a) CH_3NO_2, DBU, μw, 15 min, 100 °C.

Figure 51. (A) X-ray crystal structure of compound **144e**. (B) X-ray crystal structure of compound **144l**.



This method was also extended to compound **145d-f** activated by a nitro group (Table 11). We observed a dramatic change in the course of the reaction, which afforded a mixture of unexpected and unknown products, that each possessed an additional methylene group. These were separated by column chromatography. The mass spectrometry [MS *m/z* (**API-ES**): found $(M+H)^+$ 402], and the ^1H and ^{13}C spectra led us to hypothesize the formation of compounds **149**, **150**, and **151** (Table 11) (Figure 53, and 54). The structural confirmation of **149a** and **149b** was conducted by single-crystal X-ray diffraction (Figure 52A and 52B).

Table 11. Microwave-assisted nitromethane addition to **145d-f**.



145d-f | **149a** **150a** **151a** | **149b** **150b** **151b**

Key. a) CH_3NO_2, DBU, μw, 15 min, 100 °C

Entry	Substrate	Product	Yield	Product	Yield
1	**145d**	**149a**	29%	**149b**	18%
2	**145e**	**150a**	12%	**150b**	35%
3	**145f**	**151a**	10%	**151b**	20%

Figure 52. (A) X-ray crystal structure of compound **149a**. (B) X-ray crystal structure of compound **149b**.

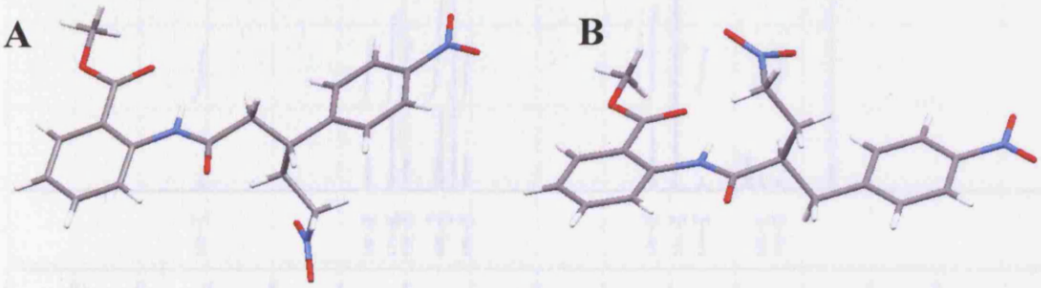


Figure 53. The ^{1}H NMR ($CDCl_3$) spectrum of **149a**.

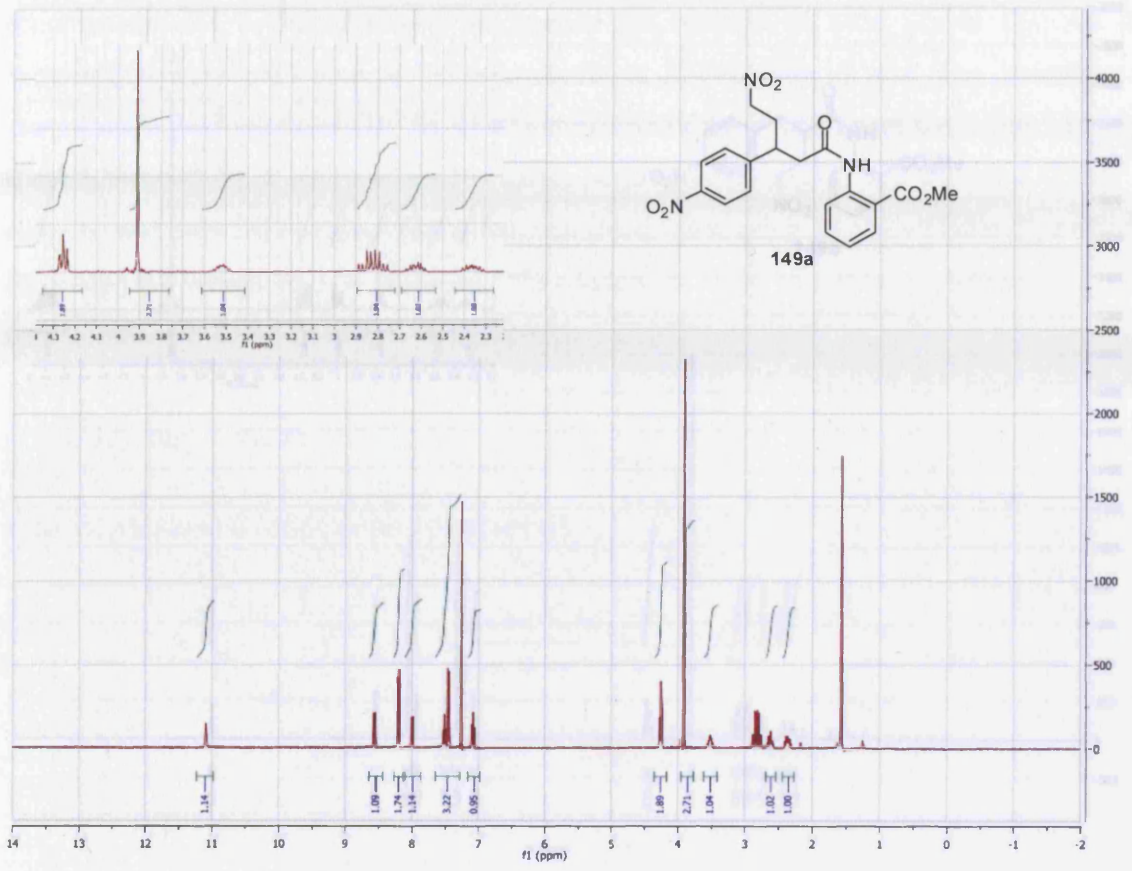
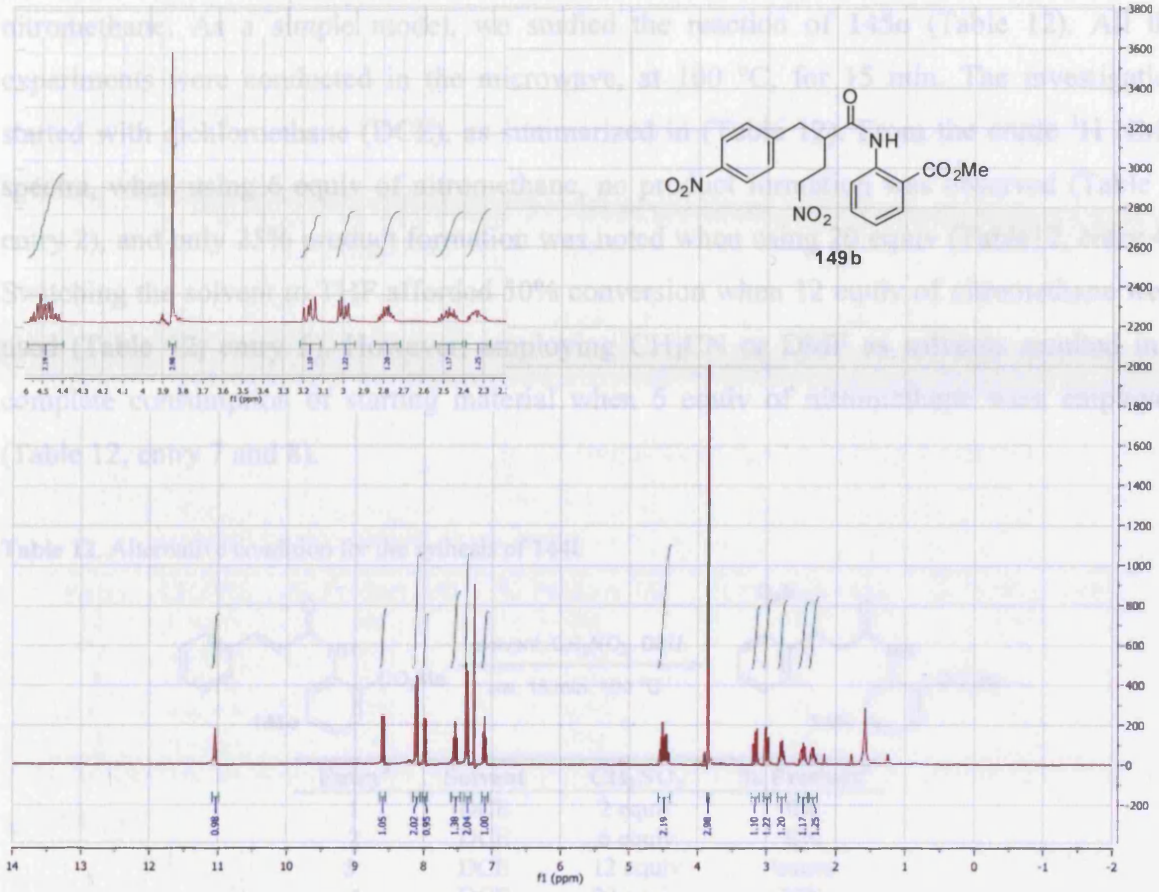
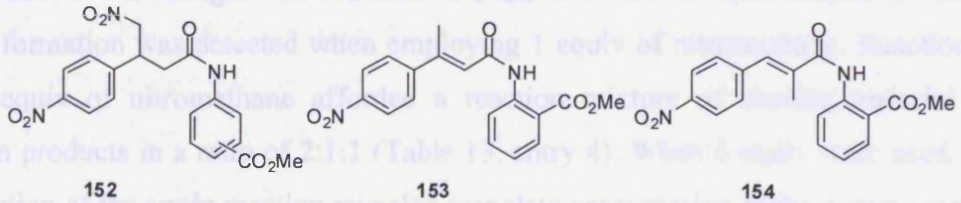


Figure 54. The ^{1}H NMR ($CDCl_3$) spectrum of **149b**.



During our synthesis we neither isolated the nitroderivative **152** nor detected its formation (Figure 55). However, from the reaction of **145d**, we isolated an unexpected product (approximately 5%), whose structure we hypothesized to be either **153** or **154** (Figure 55).

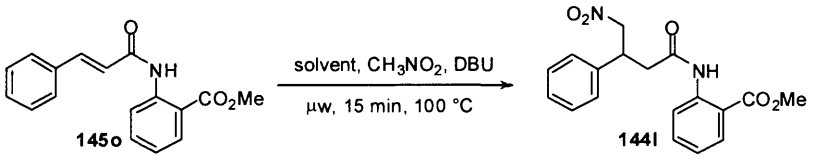
Figure 55. Potential intermediates of the conjugate addition of nitromethane to **145d-f**.



Repetition of the reaction of **145d** with nitromethane in presence of DBU, in nitromethane at room temperature provided the same product mixture. Due to higher reactivity of the substrate **145d** toward the conjugate addition, we observed the total consumption of the starting material after 12 h. To investigate whether the formation of **152** was possible without an excess of nitromethane, and to gain an insight into the reaction mechanism, we addressed

the possibility of performing the Michael addition in different solvent media, using less nitromethane. As a simple model, we studied the reaction of **145o** (Table 12). All the experiments were conducted in the microwave, at 100 °C, for 15 min. The investigation started with dichloroethane (DCE), as summarized in (Table 12). From the crude ^{1}H NMR spectra, when using 6 equiv of nitromethane, no product formation was observed (Table 5, entry 2), and only 25% product formation was noted when using 20 equiv (Table12, entry 4). Switching the solvent to THF afforded 50% conversion when 12 equiv of nitromethane were used (Table 12, entry 5). However, employing CH_3CN or DMF as solvents resulted in a complete consumption of starting material when 6 equiv of nitromethane were employed (Table 12, entry 7 and 8).

Table 12. Alternative condition for the sythesis of **144l**.

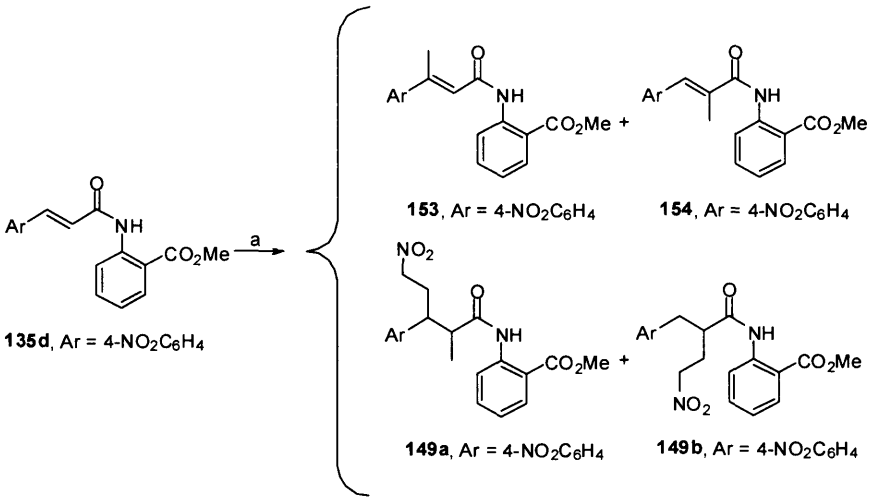


Entry[a]	Solvent	CH_3NO_2	% Product[b]
1	DCE	2 equiv	0%
2	DCE	6 equiv	0%
3	DCE	12 equiv	traces
4	DCE	20 equiv	25%
5	THF	12 equiv	50%
6	CH_3CN	3 equiv	50%
7	CH_3CN	6 equiv	100%
8	DMF	6 equiv	100%

a) All the experiments were conducted at 0.8 M concentration. b) The conversion was determined by ^{1}H NMR spectroscopy.

We proceeded to investigate the reaction of **145d** in DMF. As summarized in Table 13, no product formation was detected when employing 1 equiv of nitromethane. Reaction of **145d** with 3 equiv of nitromethane afforded a reaction mixture of starting material and two unknown products in a ratio of 2:1:1 (Table 13, entry 4). When 6 equiv were used, ^{1}H NMR investigation of the crude reaction revealed complete consumption of the starting material and the presence of two unknown products, compound **149a** and **149d** in a ratio of 2:2:1:1. Purification by column chromatography afforded a 1:1 mixture of the two new isomeric products in 17% yield. Their structures were hypothesized to be derivatives **153** and **154** by analysis of the ^{1}H NMR spectra and mass spectrometry of the mixture. Formation of **152** was never detected during these experiments (Table 13).

Table 13. Product distribution of reaction of **145d**.



Key a) DMF, CH_3NO_2, DBU, μw, 15 min, 100 °C.

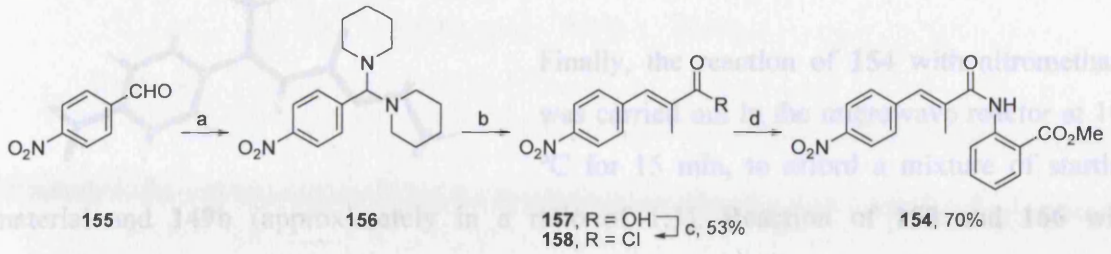
Entry	CH_3NO_2	% Product 153	% Product 154	% Product 149a	% Product 149b
1	0 equiv	0%	0%	0%	0%
2	1 equiv	10%	10%	0%	0%
3	3 equiv	25%	25%	0%	0%
4	4 equiv	40%	40%	0%	0%
5	6 equiv	30%	30%	20%	20%

a) All the experiments were conducted at 0.8 M concentration. b) The conversion was determined by ^{1}H NMR spectroscopy.

The reaction of the isomeric mixture of **153** and **154** with nitromethane, in presence of DBU, carried out at 100°C, for 15 min in the microwave, afforded a mixture of compounds **154**, **149a** and **149b** (approximately ratio 1:2:1). These results strongly suggested that **153** and **154** were the key reactive intermediates undergoing conjugate addition of nitromethane to form **149a** and **149b**.

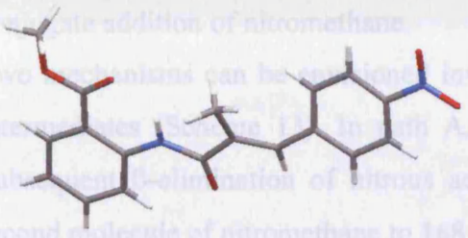
With these results in hand, we turned our attention to the structure confirmation of the compounds **153** and **154**, by preparing them through the routes depicted in Scheme 11 and 12, respectively. A fast method for the preparation of aminal[380] **156** has been reported from the reaction of aromatic aldehyde **155** and piperidine in the presence of potassium carbonate. The aminal **156** could be easily converted to the carboxylic acid **157** in reaction with methylmalonic in presence of pyridine[381] (Scheme 11). Chlorination with thionyl chloride,[375] followed by amide coupling with 2-methyl anthranilate provided compound **154**. The assignment of the *trans* configuration in **154** derives from NOE measurement at 400 MHz in $CDCl_3$. nOe experiments did not show a correlation between the 2-methyl group and vinyl hydrogen of the α,β unsaturated system. The stereochemical determination of **154** was confirmed by single-crystal X-ray diffraction (Figure 56).

Scheme 11. Synthetic route to **154**.



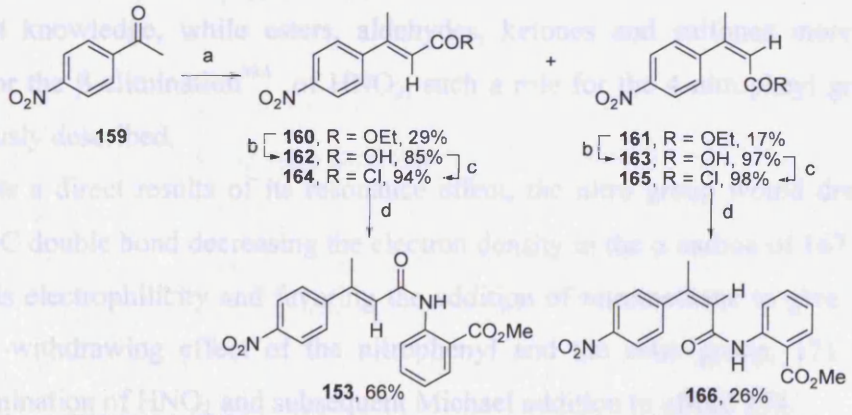
Key: a) K_2CO_3, piperidine, Ar, rt, overnight; b) Pyridine, $CH(CH_3)(CO_2H)_2$, Ar, 100 °C, 1h; c) $SOCl_2$, toluene, Ar, reflux, 2h; d) DCM, pyridine, 2-methyl anthranilate, Ar, rt, overnight.

Figure 56. X-ray crystal structure of compound **154**.



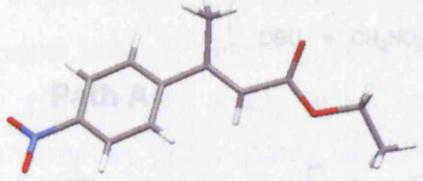
In another sequence, the Horner-Wadsworth-Emmons[382] reaction of acetophenone **159** with triethyl phosphonoacetate afforded a 1:1 mixture of the diastereoisomer **160** and **161** (Scheme 12). These were separated by column chromatography. The assignment of the *trans* configuration in **160** derives from NOE measurement at 400 MHz in $CDCl_3$. NOe experiments did not show a correlation between the 2-methyl group and vinyl hydrogen of the α,β unsaturated system. The stereochemical determination of **160** was confirmed by single-crystal X-ray structure (Figure 56). Esters **160** and **161** were saponified to the corresponding acids, and subsequently converted into the acids chloride **164** and **165**, respectively.[375] Amide coupling of the resulting acid chlorides with 2-methyl anthranilate provided **153** and **166**. Analysis and comparison of the ^1H NMR spectra revealed **153** as the compound whose formation was detected when **145d** was initially reacted with nitromethane as the solvent at 100 °C for 15 minutes in the microwave (Table 11, entry 1; Figure 55).

Scheme 12. Synthetic route to **153** and **166**.



Key: a) DME, NaH, triethylphosphonoacetate, Ar, rt, overnight; b) EtOH, KOH 1.5M, reflux, 1 h; c) toluene, $SOCl_2$, Ar, reflux, 2 h; d) DCM, pyridine, 2-methyl anthranilate, Ar, rt, overnight.

Figure 57. X-ray crystal structure of compound **160**.



Finally, the reaction of **154** with nitromethane was carried out in the microwave reactor at 100 °C for 15 min, to afford a mixture of starting material and **149b** (approximately in a ratio of 1:1). Reaction of **153** and **166** with nitromethane was carried out in the microwave at 100 °C for 15 min, to afford, as expected, **149a**, showing that the stereochemistry does not effect the reactivity **153** and **166** toward the conjugate addition of nitromethane.

Two mechanisms can be envisioned involving compounds **153** and **154** as the key reactive intermediates (Scheme 13). In path A, nitromethane adds to **167** in a conjugate fashion. Subsequent β-elimination of nitrous acid affords the olefin **168**. Conjugate addition of a second molecule of nitromethane to **168** gives **170**.

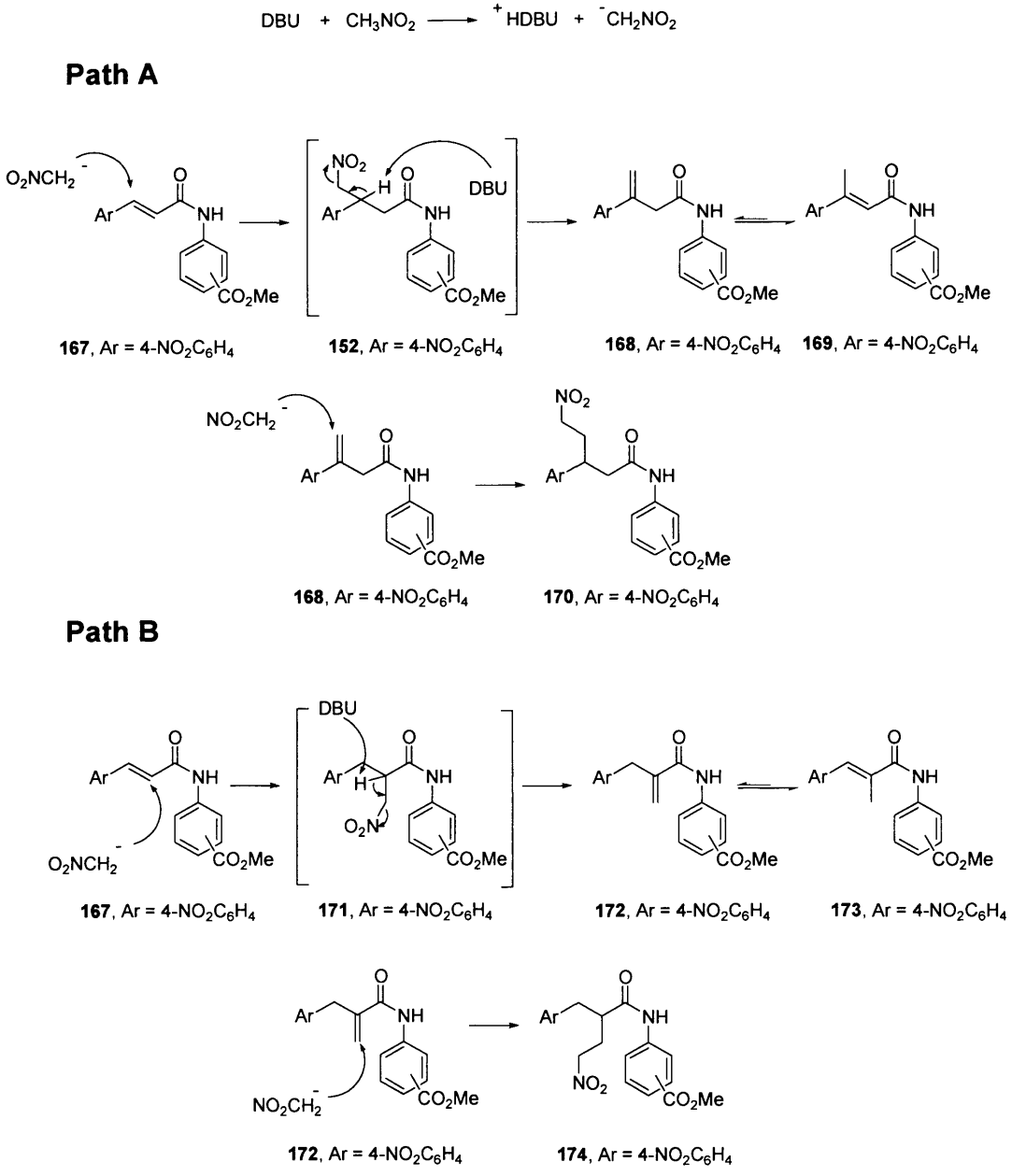
We believe that the nitrophenyl moiety is critical for the reactivity of our substrate **145** for various reasons. As direct results of the inductive effect of the nitro group, compounds **145d-f** show an increased reactivity towards the conjugate addition of nitromethane. Moreover, the acidifying effects of the p-nitrophenyl moiety (with pKa value of approximately –11.35),[383] make **152** a highly reactive species, which undergoes instant β-elimination, and play a key role in promoting the subsequent conjugate addition of nitromethane to **168**.

Preparation of olefins via ionic process from aliphatic nitro compounds has been amply described in the literature.[384] The nitro group at the β-position of an electron-withdrawing moiety readily undergoes β-elimination to afford alkenes upon treatment with base. The Michael addition of nitromethane followed by the elimination of HNO_2, has been used widely as a successful strategy in the synthesis of polyfunctionalized unsaturated carbonyl derivatives.[384]

To our best knowledge, while esters, aldehydes, ketones and sulfones more commonly employed for the β-elimination[384] of HNO_2, such a role for the 4-nitropheyl group has not been previously described.

In Path B, as a direct results of its resonance effect, the nitro group would draw electrons from the C=C double bond decreasing the electron density in the α carbon of **167** and thereby increasing its electrophilicity and favoring the addition of nitromethane to give **171**. Due to the electron-withdrawing effect of the nitrophenyl and the ester group, **171** is prone to undergo elimination of HNO_2 and subsequent Michael addition to afford **174**.

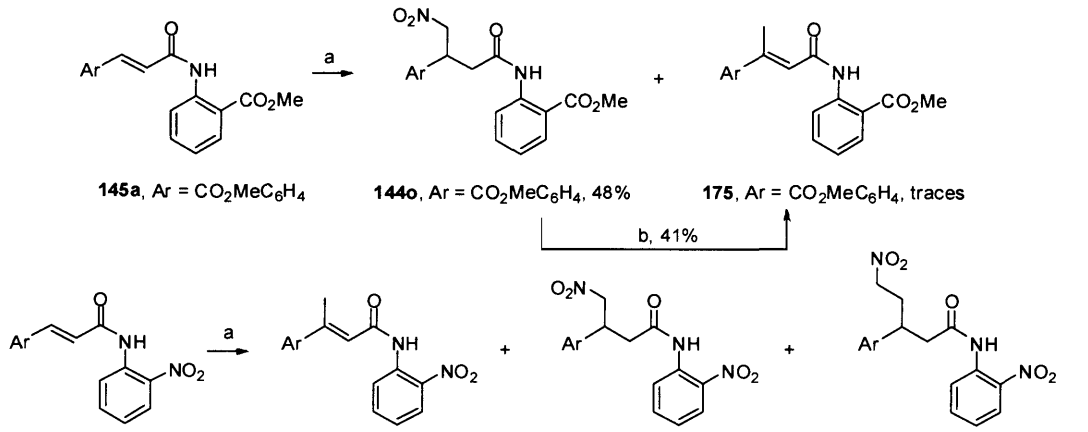
Scheme 13. Proposed mechanism for the formation of compounds **149-151**.



To probe and better understand the role of the nitro substituent, analogues of **145d** possessing alternate electron-withdrawing groups have been further explored. Replacing the nitro group in **145d** by methyl ester (**145a**) markedly reduced its reactivity toward the de-nitration-Michael addition process. Under the microwave conditions at 100 °C, reaction of **145a** with nitromethane afforded the 1,4 addition product **144o**, and traces of **175** were detected (Scheme 14). On heating at 150 °C (Scheme 14), **144o** underwent denitration to afford **175**, but no Michael addition occurred. This result suggested that the p-nitrophenyl moiety plays an important role in facilitating the β-elimination and the successive Michael addition, and

appeared to have a remarkable and unique effect on the reactivity of the α-carbon toward the conjugate addition. Moreover, we hypothesized that replacement of the methyl ester with the nitro group at the amide terminus of **145a** would propitiously activate it toward the denitration due to the strong acidifying effect of the nitro group. Even though inductive effects become less significant as the electron-withdrawing group gets further away from the negative charge, a very electronegative group such as the nitro group (twice as electron-withdrawing as the carbonyl group) might be once again critical for the reactivity of our substrate and influence the product distribution. The reaction of **145t** afforded **176** as the main product and only traces of **144p**, and **177** were detected.

Scheme 14. Product distribution of reaction of **145a** and **145t**.



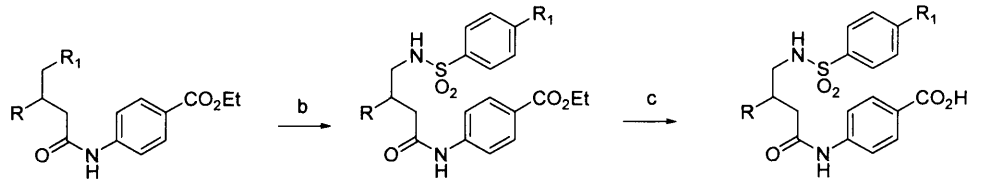
145a, Ar = $CO_2MeC_6H_4$ **144o**, Ar = $CO_2MeC_6H_4$, 48% **175**, Ar = $CO_2MeC_6H_4$, traces

145t, Ar = $CO_2MeC_6H_4$ **176**, Ar = $CO_2MeC_6H_4$, 51% **144p**, Ar = $CO_2MeC_6H_4$, traces **177**, Ar = $CO_2MeC_6H_4$, traces

Key: a) CH_3NO_2, DBU, μw, 100 °C, 15 min; b) CH_3NO_2, DBU, μw, 150 °C, 15 min.

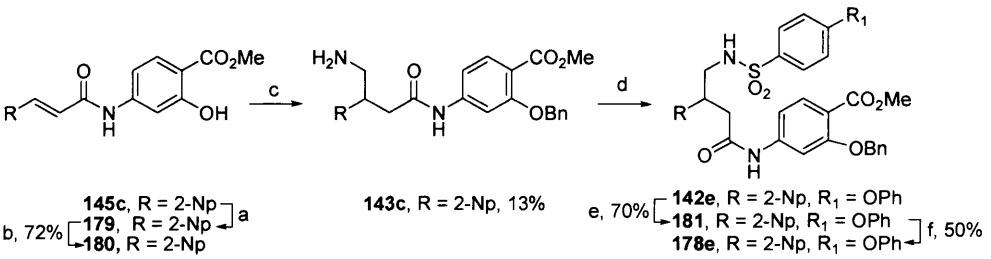
Finally, in an effort to expand the utility of our protocol to the synthesis of the T-shape scaffold, substrates **144a** and **144b** were converted into the corresponding amino compounds **143a-b** by reduction of the nitro group in presence of $NaBH_4$ and $NiCl_2$.[385] For practical reasons, the intermediates **143a-b** were not purified. Derivatization of **143a-b** under Schotten-Bauman conditions, followed by saponification afforded the final targets **178a-d** in good overall yields (Scheme 15). Compound **178e** was prepared via conjugate addition of nitromethane to the benzyl protected amide **179**. Reduction of **179** to amino compound **143c** and subsequent coupling with 4-phenoxybenzenesulfonyl chloride furnished the sulfonamide **142e**. Finally, hydrogenolysis followed by saponification afforded the final target molecule **178e** (Scheme 16).

Scheme 15. Synthesis of the T-shape molecules **178**.



Key: a) $NiCl_2$, $NaBH_4$, MeOH, THF, 0 °C, 15 min; b) 4-phenoxybenzenesulfonyl chloride or tosyl chloride, K_2CO_3, dioxane, H_2O, rt, 2-3 h; c) THF, MeOH, NaOH 1M, rt, overnight.

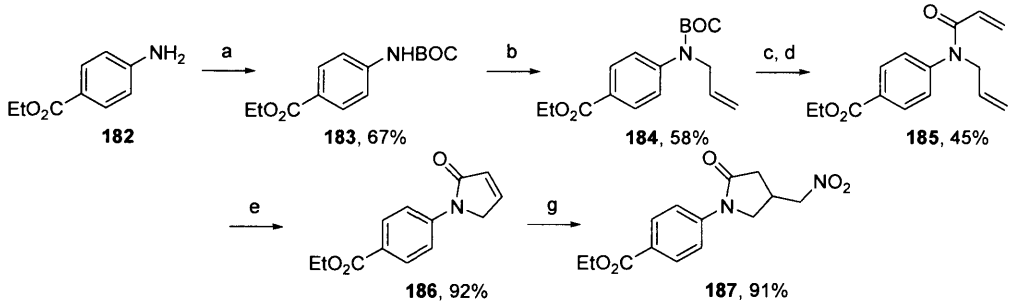
Scheme 16. Synthetic route to compound **178e**.



Key: a) DMF, K_2CO_3, benzyl bromide; b) CH_3NO_2, DBU, μw, 15 min, 100 °C; c) $NiCl_2$, $NaBH_4$, MeOH, THF, 0 °C, 15 min; d) 4-phenoxybenzenesulfonyl chloride, K_2CO_3, dioxane, H_2O, rt, 3 h; e) MeOH, ammonium formate, 10% Pd/C, H_2, 2 days; f) THF, MeOH, NaOH 1M, rt, overnight.

Our protocol provided a convenient entry to pyrrolidine **187** through the intermediacy of **186**, obtained in excellent yield by treatment of **185** under olefinic metathesis condition using the second generation Grubb's catalyst[386] (Scheme 17). The pyrrolidine moiety is a drug-like scaffold that has featured in the design of peptidomimetics and small molecules as potential pharmacologic agents for the treatment of several diseases.[387-390] In addition, the nitromethane motif of **187** constitutes a unique branching point for further diversification, and the carbonyl motif itself is an important functionality, which provides an enormous scope for molecular design allowing the introduction of structural and chemical diversity.

Scheme 17. Synthetic route to pyrrolidine **187**.

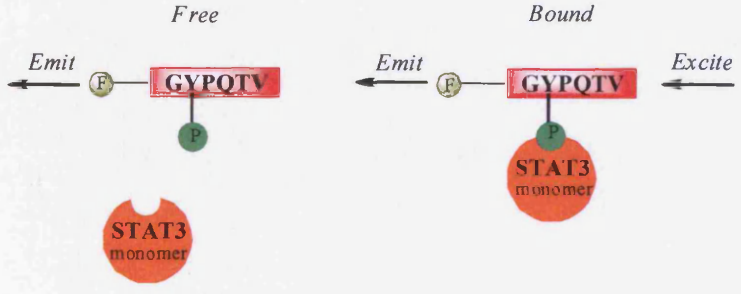


Key: a) Boc_2O, THF, Ar, reflux, 24 h; b) THF, NaH, allyl bromide, rt, 48 h; c) TFA, DCM, rt, 2 h; d) DCM, Et_3N, acryloyl chloride, Ar, rt, 4 h; e) Toluene, 2nd generation Grubb's catalyst, reflux, 1h; f) CH_3NO_2, DBU, μw, 100 °C, 15 min.

7.3 Biological results

Our primary aim was the synthesis of novel small molecules that can block STAT3 activation *in vitro* by interaction with STAT3 SH2 domain. The compounds were designed to bind to the SH2 domain of one STAT3 monomer, disrupting the dimerization. All the synthesized compounds were evaluated for their ability to disrupt active STAT3 determined by reduction in DNA binding activity, which can be measured by an electrophoretic shift assay (EMSA). Nuclear extract containing active STAT3 were incubated with different concentration of the compounds, prior to incubation with ^{32}P-labeled hSIE olinucleotide probe, and analyzed by EMSA. The ability of the compounds to disrupt active STAT3 was indirectly determined by measuring the level of STAT3-^{32}PDNA binding activity. Unfortunately, none of the synthesized compounds retained the potency of interaction with and the disruption of STAT3 activity as compared to the first hits **NSC59263** and **NSC64859**, emerged from computational analyses.

In the context of the search of inhibitors of STAT3 activity, considerable efforts have been directed in our laboratories to the validation and establishment of a robust fluorescent polarization assay (FP), suitable for HTS screening, which allows screening for small molecules that directly bind to the STAT3 SH2 domain and thereby inhibit its activity. The assay has been previously described by Schust and coworkers.[391] The basis of this assay is the binding of a fluorescein-labeled phosphopeptide (GY*PQTV) derived from the interleukin-6 receptor subunit gp130 to unphosphorylated STAT3 with K_d of 150 nM. The fluorescence polarization is a powerful technique and more accurate then electrophoretic methods such as EMSA.



Briefly, fluorescent polarization measurements are based on the assessment of the rotational motion of fluorescently labeled macromolecules. The fluorophore attached to the small binding partner (e.g. GY*PQTV peptide) is exited by polarized light and the rotational speed of a molecule in solution inversely correlates with its effective molecular weight. Therefore, the fluorescence polarization of the unbound small binding partner will be low, and its binding to a larger binding partner (e.g. STAT3 monomer) will increase the polarization of the emitted fluorescence. In summary, the assay will allow us the direct analysis of the ability of our library of compounds to bind the STAT3 SH2 domain.

7.4 Conclusion

In the search for novel small molecule disrupters of STAT3 activity, a convenient microwave assisted conjugate addition of nitromethane to compounds **145** has been developed. The generality and applicability to a broad range of substrate makes the reaction valuable to provide useful nitrogen containing intermediates for the synthesis of druglike molecules such as **178** and **187**. Moreover, the presence of the nitro group at the cinnamic acid-terminus of **145d-f** proved to be crucial for its unique reactivity toward the conjugate addition of nitromethane.

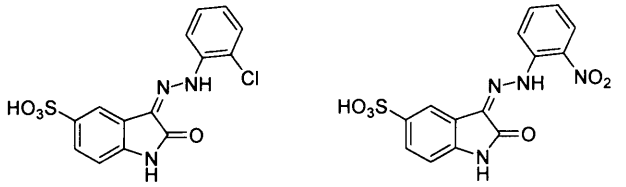
Chapter 8

8.0 Synthesis of oxindole derivatives as potential inhibitors of Aurora kinases and SHP-2 phosphatase

8.1 Introduction

As part of our program toward the development of Aurora kinases and SHP-2 inhibitors, the oxindole derivative **HL10581** and **NSC117199** emerged as a lead compounds from a high throughput screen for Aurora-A and SHP-2, respectively (Figure 58). We synthesized various series of oxindole derivatives as potential inhibitors of Aurora-A and SHP-2.

Figure 58. Structure of the initial hits **HL10581** and **NSC117199**.

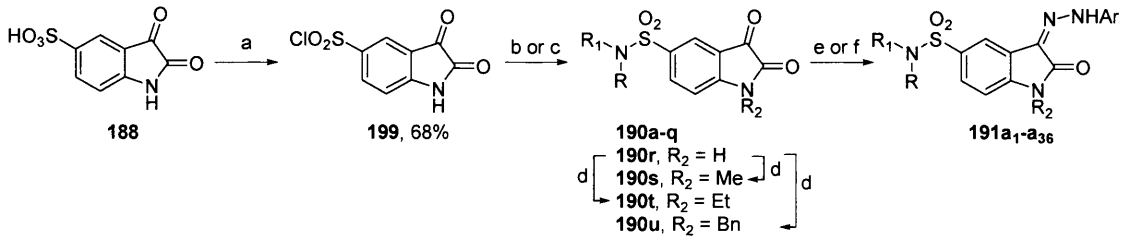


HL10581, Aurora-A, IC_{50} 1-5 μM **NSC117199**, SHP-2, IC_{50} 47 μM

8.2 Chemistry

We initially prepared the library of sulfonamides **191** aimed at probing the role of the sulfonic acid group, the effect of incorporation of hydrophilic and hydrophobic alkyl and aryl groups at the 5-postion of **HL10581** and **NCS117199**. Treatment of 5-isatinsulfonic acid sodium salt dihydrate (**188**) with $POCl_3$, in tetramethylene sulfone at 60 °C afforded the key intermediate[392] **189** (Scheme 18).

Scheme 18. Synthetic route to library **191**.



Key: a) $POCl_3$, tetramethylene sulfone, 60°C , 4 h; b) $NHRR_1$, DIPEA, THF, rt, overnight; c) morpholine, DCM, Ar, rt, 3 h; d) Ethyl bromide or methyl iodide or benzyl bromide, DMF, NaH, Ar, rt, overnight; e) $ArNHNH_2$, HCl 4M, EtOH, reflux, 5 h; f) $ArNHNH_2$, HCl 4M, EtOH, μw, 120 °C , 15 min.

Scheme 18 outlines the conditions followed for the synthesis, of the sulfonamides **190a-u**, according to a procedure previously reported by Lee and coworkers[392] (Table 14). *N*-methyl (**190s**), *N*-ethyl (**190t**), and *N*-benzyl (**190u**) derivatives were synthesized by reaction of the

parent compound **190r** with NaH, followed by alkylation with the appropriate halides (Table 14). Compounds **191a$_1$-a$_3$,a$_6$,a$_{19}$-a$_{20}$** were prepared by condensation of the amide precursors **190a,b,j-p** with the commercial 2-nitrophenylhydrazine according to a protocol developed by Kuyper and coworkers of GlaxoSmithKline[393] (Scheme 18, Table 15). We found that the condensation can be effected by the microwave heating, shortening significantly the reaction time to 15 min, and therefore meeting our need of a straightforward synthetic route to the target molecules (Scheme 18). NMR spectroscopy was used for determining the purity the compounds. HPLC methods (typically two methods) were also developed to assess the purity of the most active compounds. Ensuring the quality of the compound collection is important to minimize both false positive and false negative biological data and to improve data quality.

Table 14. Synthesis of intermediates **190**.

Entry	R	R_1	R_2	Yield 189 → 190
190a	H	H	H	47%
[a]**190b**	H	*N,N*-Dimethylethyl	H	-
190c	H	Propyl	H	45%
190d	H	*Iso*-propyl	H	70%
190e	H	2-Methoxyethylyl	H	66%
190f	H	*Sec*-butyl	H	30%
[a]**190g**	H	Morpholinyl	H	-
190h	H	Tetrahydrofurfuryl	H	66%
190i	H	Furfuryl	H	95%
190j	H	2-Thiophenemethyl	H	49%
190k	H	3-Methoxybenzyl	H	54%
190l	H	4-Methoxybenzyl	H	25%
190m	Me	Benzyl	H	47%
[a]**190n**	H	4-(Aminomethyl)pyridyl	H	-
190o	H	2-(Aminomethyl)pyridyl	H	58%
190p	H	4-Chlorobenzyl	H	57%
190q	H	Benzyl	H	64%
190r	Me	Me	H	68%
190s	Me	Me	Me	67%
190t	Me	Me	Et	82%
190u	Me	Me	Benzyl	29%

a) The crude material was used in the next step without further purification.

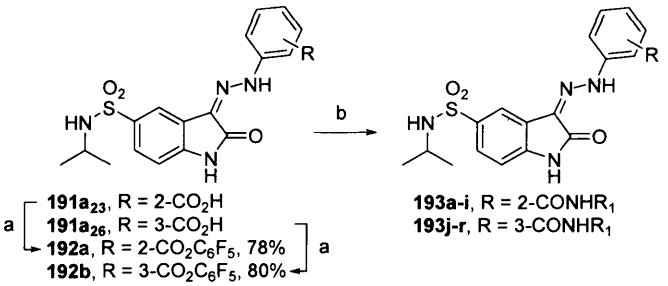
Table 15. Synthesis of hydrazones **191**.

Entry	R	R_1	R_2	Ar	Yield 190 →191
191a$_1$	H	H	H	2-$NO_2C_6H_4$	58%
191a$_2$	Me	Me	H	2-$NO_2C_6H_4$	60%
191a$_3$	Me	Me	Me	2-$NO_2C_6H_4$	36%
191a$_4$	Me	Me	Et	2-$NO_2C_6H_4$	63%
191a$_5$	Me	Me	Benzyl	2-$NO_2C_6H_4$	21%
191a$_6$	H	*N,N*-Dimethylethyl	H	2-$NO_2C_6H_4$	24%
191a$_7$	H	Propyl	H	2-$NO_2C_6H_4$	56%
191a$_8$	H	*Iso*-propyl	H	2-$NO_2C_6H_4$	50%
191a$_9$	H	2-Methoxyethyl	H	2-$NO_2C_6H_4$	49%
191a$_{10}$	H	*Sec*-butyl	H	2-$NO_2C_6H_4$	50%
191a$_{11}$	H	Morpholinyl	H	2-$NO_2C_6H_4$	56%
191a$_{12}$	H	Tetrahydrofurfuryl	H	2-$NO_2C_6H_4$	63%
191a$_{13}$	H	Furfuryl	H	2-$NO_2C_6H_4$	86%
191a$_{14}$	H	2-Thiophenemethyl	H	2-$NO_2C_6H_4$	59%
191a$_{15}$	H	3-Methoxybenzyl	H	2-$NO_2C_6H_4$	45%
191a$_{16}$	H	4-Methoxybenzyl	H	2-$NO_2C_6H_4$	57%
191a$_{17}$	Me	Benzyl	H	2-$NO_2C_6H_4$	54%
191a$_{18}$	H	4-(Aminomethyl)pyridyl	H	2-$NO_2C_6H_4$	38%
191a$_{19}$	H	2-(Aminomethyl)pyridyl	H	2-$NO_2C_6H_4$	58%
191a$_{10}$	H	4-Chlorobenzyl	H	2-$NO_2C_6H_4$	43%
191a$_{21}$	H	*Iso*-propyl	H	C_6H_5	48%
191a$_{22}$	H	*Iso*-propyl	H	1-Np	48%
191a$_{23}$	H	*Iso*-propyl	H	2-$CO_2HC_6H_4$	68%
191a$_{24}$	H	H	H	2-CO_2HC_6H	30%
191a$_{25}$	H	4-Chlorobenzyl	H	2-CO_2HC_6H	66%
191a$_{26}$	H	*Iso*-propyl	H	3-$CO_2HC_6H_4$	57%
191a$_{27}$	H	H	H	3-$CO_2HC_6H_4$	58%
191a$_{28}$	H	4-Chlorobenzyl	H	3-$CO_2HC_6H_4$	55%
191a$_{29}$	H	*Iso*-propyl	H	4-$CO_2HC_6H_4$	75%
191a$_{30}$	H	H	H	4-$CO_2HC_6H_4$	53%
191a$_{31}$	H	4-Chlorobenzyl	H	4-$CO_2HC_6H_4$	77%
191a$_{32}$	H	H	H	3-$NO_2C_6H_4$	49%
191a$_{33}$	H	4-Chlorobenzyl	H	3-$NO_2C_6H_4$	68%
191a$_{34}$	H	H	H	4-$NO_2C_6H_4$	59%
191a$_{35}$	H	4-Chlorobenzyl	H	4-$NO_2C_6H_4$	74%
191a$_{36}$	H	Benzyl	H	2-$NO_2C_6H_4$	74%

Compounds **191a$_{23}$** and **191a$_{26}$** emerged as potent inhibitors of SHP-2 from the screening of library **191**. The carboxylic acid moiety allowed us to introduction of structural and chemical diversity on phenylhydrazone moiety of **191a$_{23}$** and **191a$_{26}$** generating a new library of analogues **193** (Table 16) and further probing the role of the carboxylic acid.

Table 16 illustrates the general method for the synthesis of the carboxamides **193a-t**. The synthetic strategy, previously described by Bramson *et al.*,[394] relies upon the conversion of the acids **191a$_{23}$** and **191a$_{26}$** into the corresponding esters **192a** and **192b** by treatment with pentafluorophenyl trifluoroacetate. Coupling of the activated esters **192a** and **192b** with a range of primary and secondary amine under very mild conditions afforded the desired library **193** (Table 16).

Table 16. Synthesis of amides **193**.



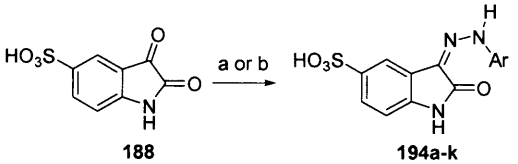
Key: a) Pentafluorophenyl trifluoroacetate, pyridine, DMF, rt, Ar, 1.5 h; b) $NHRR_1$, pyridine, CH_3CN, rt, Ar, overnight.

Entry	R = 2-CONHR$_1$, R$_1$ =	Yield	Entry	R = 3-CONHR$_1$, R$_1$ =	Yield
193a	Furfuryl	47%	**193j**	Furfuryl	77%
193b	H	38%	**193k**	H	54%
193c	Dimethylaminoethyl	37%	**193l**	Dimethylaminoethyl	39%
193d	2-Methoxyethyl	49%	**193m**	2-Methoxyethyl	46%
193e	Benzyl	84%	**193n**	Benzyl	70%
193f	2-(Aminomethyl)pyridyl	37%	**193o**	2-(Aminomethyl)pyridyl	70%
193g	2-Morpholin-4-yl-ethyl	67%	**193p**	2-Morpholin-4-yl-ethyl	57%
193h	Me	69%	**193q**	Me	79%
193i	Et	75%	**193r**	Et	72%

We also studied the effects due to the replacement of the chlorine and nitro group of **HL10581** and **NSC117199,** obtaining a library of novel isatins **194** (Table 17). The preparation of library **194** involved one-step condensation of **188** with a range of commercially available aromatic hydrazines in 30-93% yields (Table 17) and high purity. In order to overcome the poor solubility of the 5-isatinsulfonic acid sodium salt (**188**) in ethanol, the reactions were carried out using aqueous HCl as co-solvent. Once again, microwave technology provided an efficient alternative to conventional heating allowing us to generate the desired library **184** in a combinatorial fashion.

Table 17. Synthesis of hydrazones **194**.

Entry	Ar	Yield
194a	C_6H_5	87%
194b	2-MeC_6H_4	75%
194c	2,6-$Cl_2C_6H_3$	78%
194d	2-EtC_6H_4	30%
194e	2-FC_6H_4	93%
194f	2-$CF_3C_6H_4$	87%
194g	C_6F_5	51%
194h	1-Np	78%
194i	2,4-$Cl_2C_6H_3$	69%
194j	2,5-$Cl_2C_6H_3$	76%
194k	2-$CO_2HC_6H_4$	82%



188 **194a-k**

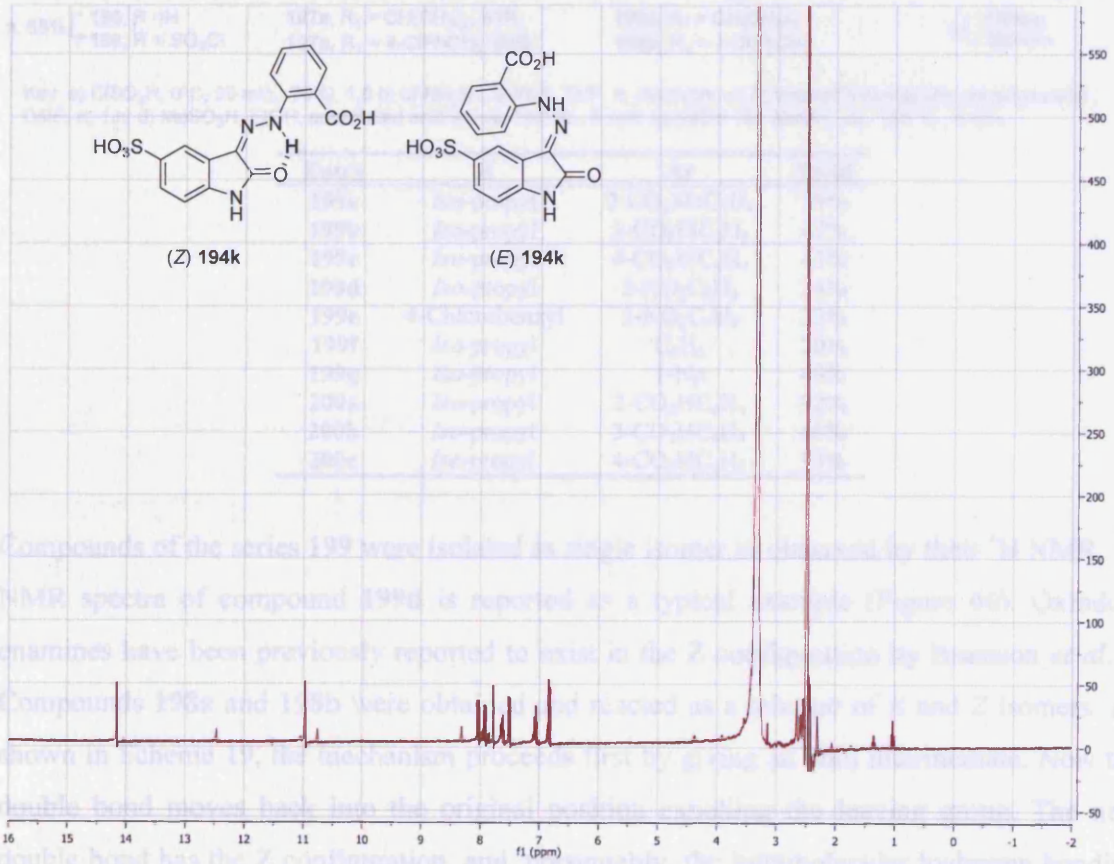
Key: a) $ArNHNH_2$, HCl 4M, EtOH, μw, 120 °C, 15 min; b) 2-(CO_2H)phenyl hydrazine, HCl 4M, EtOH, μw, 150 °C, 5 min

Compounds in the series **191**, **193** were generally isolated as single isomer. Unfortunately, we have not been able to confirm this stereochemistry by X-ray crystallography. However, isatin

hydrazones have been previously reported[394] to exist in the Z configuration, which is favored due to the intramolecular hydrogen bonding between the NH of the hydrazone and the carbonyl group of the oxindole.

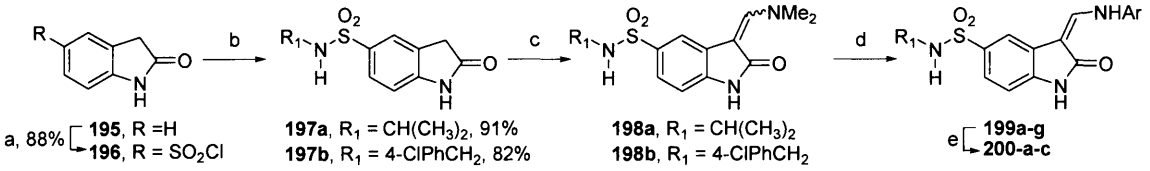
As viewed by ^{1}H NMR (Figure 59), when isatin **188** was reacted with 2-hydrazinobenzoic acid at 120 °C for 15 minutes, compound **194k** was obtained as an unexpected mixture of *E* (minor) and *Z* (major) isomers. Performing the reaction under conventional heating afforded the same isomeric mixture. Due to its poor solubility in a wide range of organic solvent, purification of **194k** by recrystallization or trituration proved to be difficult. To our delight, we found that **194k** could be obtained and isolated as a single *Z* isomer by modifying the reaction condition (microwave heating, 150 °C, 5 min). The unusual reactivity of the 2-carboxyphenyl hydrazine may be due to the presence of the *ortho* carbonyl group competing with the carbonyl of the oxindole in hydrogen bonding the NH of the hydrazone, and therefore leading to a mixture of two isomers.

Figure 59. ^{1}H NMR (DMSO-d_6) of the isomeric mixture of *Z* and *E* **194k**.



Subsequently, a focused library of enamines **199** and **200** was also prepared in order to assess the requirement of the hydrazone linker for optimal activity of derivatives **181**. The synthetic route for the synthesis of **199** and **200** is depicted in Table 18. The 5-chlorosulfonyloxindole **196** was prepared by treating the unsubstituted oxindole **195** with chlorosulfonic acid[395] at 70 °C. Reaction of **196** with isopropylamine and 4-chlorobenzylamine afforded sulfonamides **197a** and **197b**, respectively, in excellent yield and without the need for further purification. Treatment of **197a** and **197b** with *N,N*-dimethylformamidedimethylacetal in DMF at room temperature followed by condensation of the resulting dimethylaminomethyleneoxindole **188a** and **188b** with the appropriate aniline produced the required library **199** (Table 18). Finally, hydrolysis of **199a-g** proceeded smoothly under microwave heating to give the corresponding acid **200a-c** (Table 18).

Table 18. Synthesis of enamines **199** and **200**.



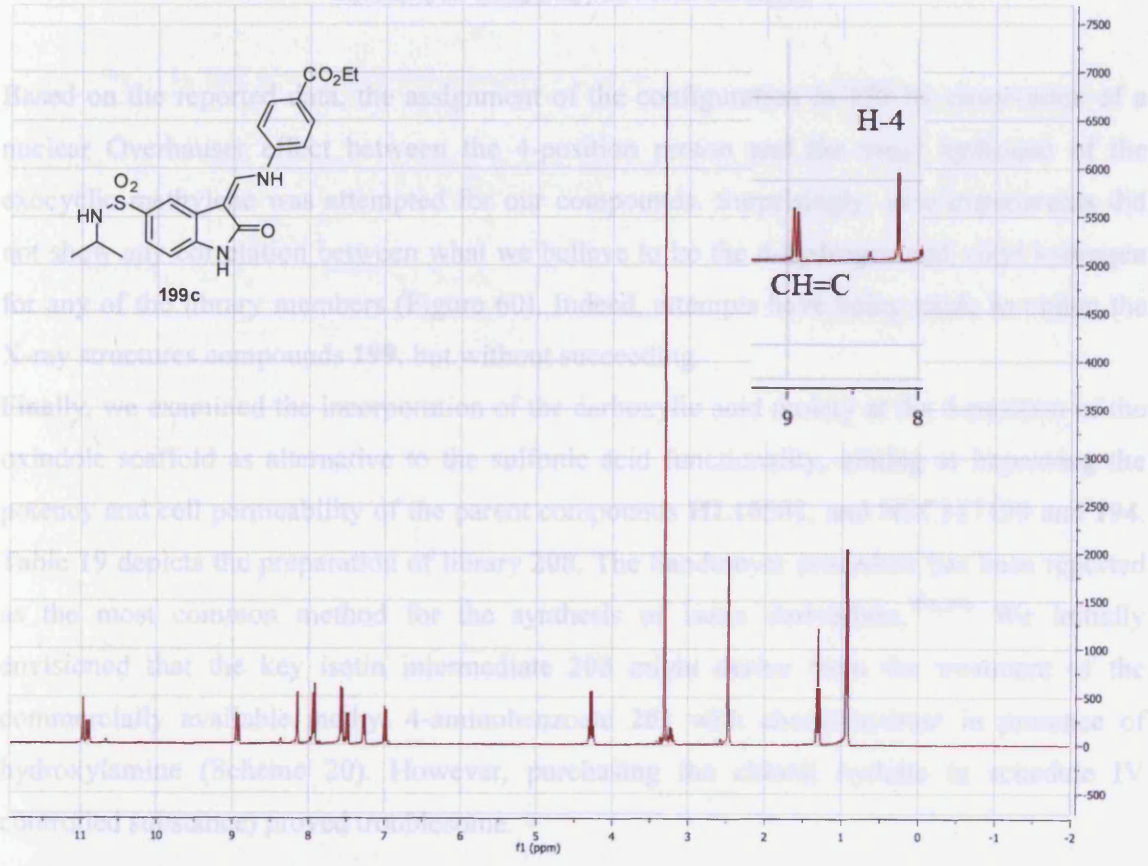
Key: a) $ClSO_3H$, 0°C, 30 min, 70°C, 1.5 h; b) NH_2R_1, DIPEA, THF, rt, overnight; c) *N,N*-dimethylformamide dimethylacetal, DMF, rt, 1 h; d) $MeSO_3H$, EtOH, substituted aniline, μw, 150 °C , 5 min; e) NaOH 1M, MeOH, μw, 150 °C , 5 min.

Entry	R	Ar	Yield
199a	*Iso*-propyl	2-$CO_2MeC_6H_4$	59%
199b	*Iso*-propyl	3-$CO_2EtC_6H_4$	47%
199c	*Iso*-propyl	4-$CO_2EtC_6H_4$	43%
199d	*Iso*-propyl	2-$NO_2C_6H_4$	34%
199e	4-Chlorobenzyl	2-$NO_2C_6H_4$	33%
199f	*Iso*-propyl	C_6H_5	70%
199g	*Iso*-propyl	1-Np	49%
200a	*Iso*-propyl	2-$CO_2HC_6H_4$	92%
200b	*Iso*-propyl	3-$CO_2HC_6H_4$	40%
200c	*Iso*-propyl	4-$CO_2HC_6H_4$	33%

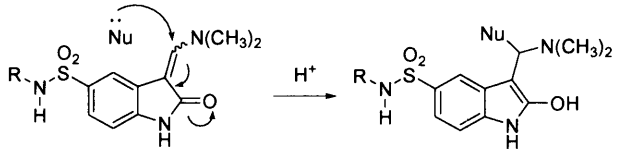
Compounds of the series **199** were isolated as single isomer as observed by their ^1H NMR. ^1H NMR spectra of compound **199d** is reported as a typical example (Figure 60). Oxindole enamines have been previously reported to exist in the Z configuration by Bramson *et al.*[394] Compounds **198a** and **198b** were obtained and reacted as a mixture of *E* and *Z* isomers. As shown in Scheme 19, the mechanism proceeds first by giving an enol intermediate. Now the double bond moves back into the original position expelling the leaving group. The new double bond has the Z configuration, and, presumably, the intramolecular hydrogen bonding between the NH of the hydrazone and the carbonyl group of the oxindole is the key interaction determining the stereochemistry (Scheme 19).

Bramson[394] and coworkers reported that confirmation of the stereochemistry of their library of enamines was achieved by observation of a nuclear Overhauser effect between the 4-position proton of the oxindole ring system and the vinyl hydrogen of the exocyclic methylene for 5-substituted oxindole derivatives. Only in some analogs where a hydrogen bond acceptor (O or N) was introduced at the 4-position of the oxindole ring, allowing the formation of an alternative hydrogen bond donor with the linker NH in the E configuration, compounds were collected as a mixture of E and Z isomers as observed by ^{1}H NMR.

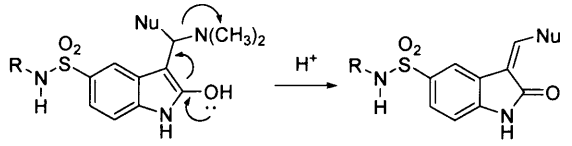
Figure 60. ^{1}H NMR (DMSO-d_6) of compound **199c**.



Scheme 19. Mechanism of the conjugate substitution.



formation of the enol intermediate

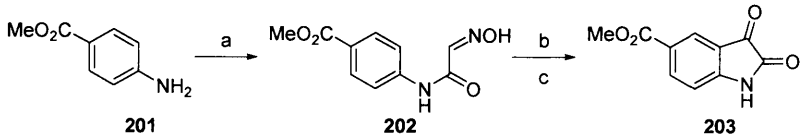


expulsion of the leaving group from the enol intermediate

Based on the reported data, the assignment of the configuration in **199** by observation of a nuclear Overhauser effect between the 4-position proton and the vinyl hydrogen of the exocyclic methylene was attempted for our compounds. Surprisingly, nOe experiments did not show any correlation between what we believe to be the 4-hydrogen and vinyl hydrogen for any of the library members (Figure 60). Indeed, attempts have being made to obtain the X-ray structures compounds **199**, but without succeeding.

Finally, we examined the incorporation of the carboxylic acid moiety at the 5-position of the oxindole scaffold as alternative to the sulfonic acid functionality, aiming at improving the potency and cell permeability of the parent compounds **HL10581**, and **NSC117199** and **194**. Table 19 depicts the preparation of library **208**. The Sandmeyer procedure has been reported as the most common method for the synthesis of isatin derivatives.[394,396] We initially envisioned that the key isatin intermediate **203** might derive from the treatment of the commercially available methyl 4-aminobenzoate **201** with choral hydrate in presence of hydroxylamine (Scheme 20). However, purchasing the chloral hydrate (a schedule IV controlled substance) proved troublesome.

Scheme 20. The Sandmeyer synthesis.



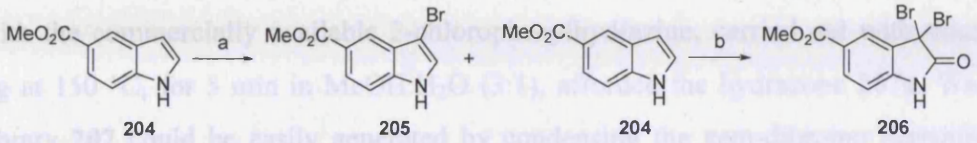
201 **202** **203**

Key: a) $Cl_3CCH(OH)_2$, $H_2NOH{\cdot}HCl$, Na_2SO_4; b) H_2SO_4; c) H_2O.

An alternative approach was adopted, providing an efficient access to the series **207**, **208**. The synthesis of **203** began methyl indole-5-carboxylate (**204**) (Scheme 21) following a literature

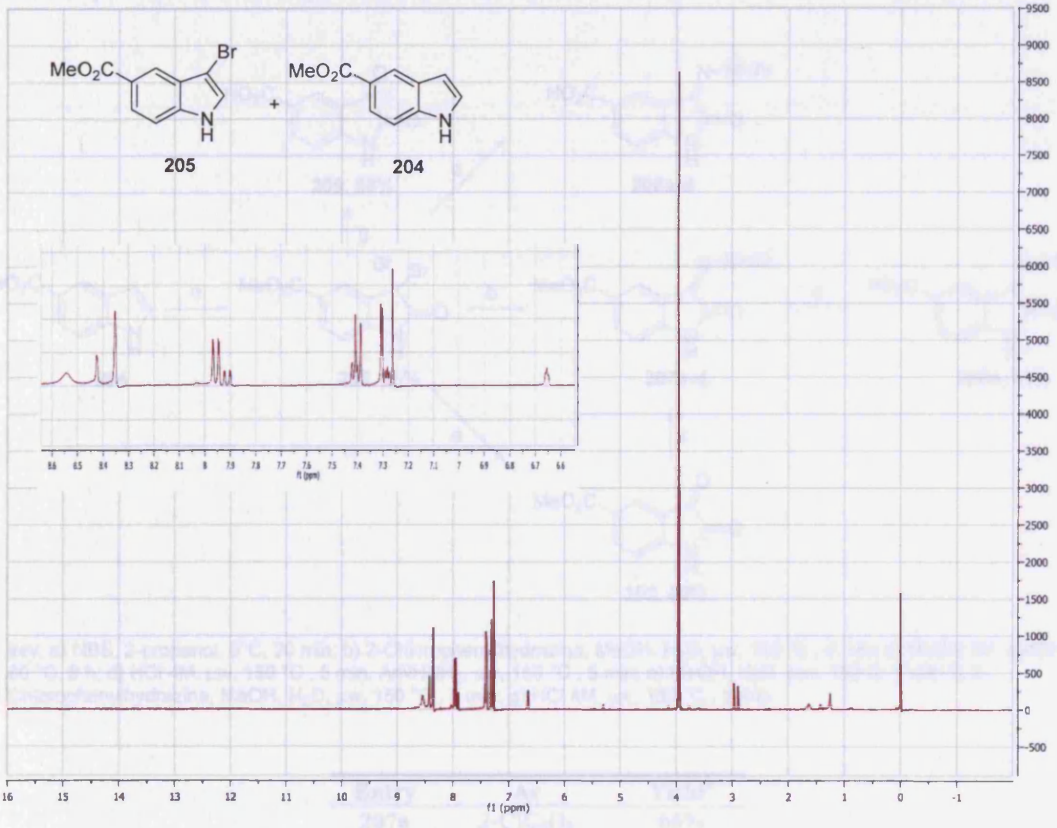
reported procedure.[397] The reaction of **204** with Br_2 in DMF afforded a crude mixture of **205** and **204** in a ratio of 3:1 (Figure 61). Treatment of the crude mixture with 1 equivalent NBS in 2-propanol-H_2O (95:5) afforded a crude mixture of **205** and the gem-dibromo derivative **206** (Figure 62) suggesting that the complete conversion of **204** into **206** could be directly accomplished by treatment with NBS.

Scheme 21. Literature reported procedure for the synthesis of **206**.



Key: a) Br_2, DMF, rt, overnight; b) NBS, 2-propanol, 0°C, 30 min.

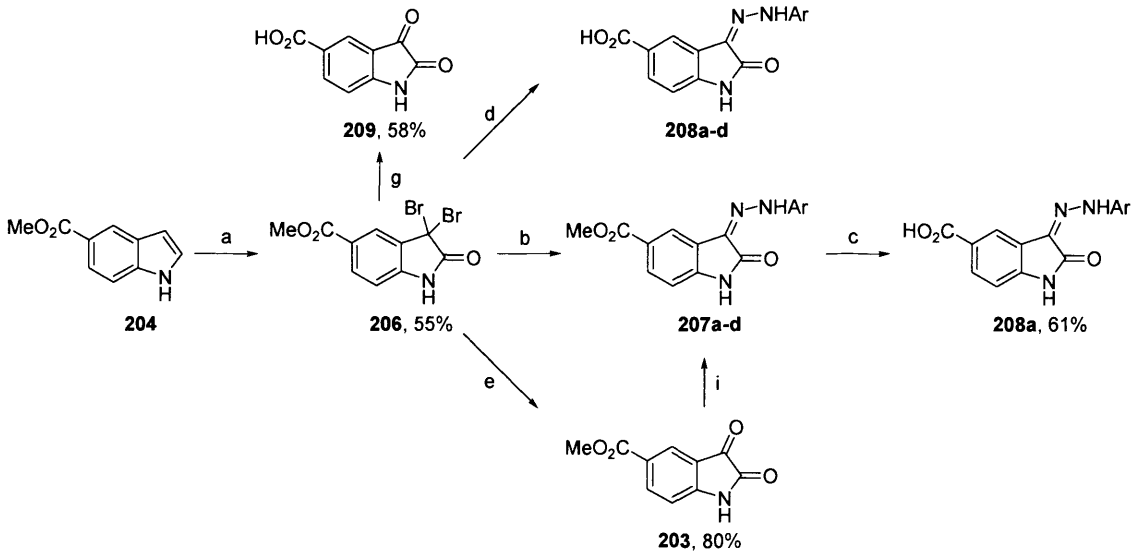
Figure 61. The ^1H NMR (DMSO-d_6) spectrum of crude **205** from reaction of **204** with Br_2 in DMF.



Conditions involving the direct use of NBS for the synthesis of gem-dibromo derivatives from the corresponding indoles have been previously reported in the literature.[397-399] However, the preparation of analogues of **206** in this manner is often complicated by the concomitant bromination of the aromatic ring depending on the substituents on the aromatic

ring itself. We believed that in our specific case the electron-withdrawing nature of the carboxylic ester group at the 5-position of **204**, might propitiously deactivate the aromatic ring toward the bromination. This hypothesis proved to be correct and compound **206** could be prepared by reacting the 5 methyl indole-5-carboxylate (**204**) with 4 equivalent of NBS in 2-propanol-H_2O (95:5) without detecting the formation of any side-products deriving from the bromination of the aromatic ring (Scheme 21) (Figure 63). Isatin **203** could be obtained by hydrolysis of **206** [microwave heating, MeOH:H_2O (3:1), 150 °C, 5 min]. Condensation of **203** with the commercially available 2-chlorophenylhydrazine, carried out with microwave heating at 150 °C, for 5 min in MeOH:H_2O (3:1), afforded the hydrazone **207a**. We found that library **207** could be easily generated by condensing the gem-dibromo derivative **206** with a range of aryl hydrazines under microwave conditions (Scheme 8, step b) (Table 19).

Table 19. Synthetic route to compounds **203**, **207**, **208**, **209**.



key: a) NBS, 2-propanol, 0°C, 30 min; b) 2-Chlorophenylhydrazine, MeOH, H_2O, μw, 150 °C , 5 min; c) NaOH 1M, MeOH, 80 °C, 8 h; d) HCl 4M, μw, 150 °C , 5 min, $ArNHNH_2$, μw, 150 °C , 5 min; e) MeOH, H_2O, mw, 150 C, 5 min; f) 2-Chlorophenylhydrazine, MeOH, H_2O, μw, 150 °C , 5 min; g) HCl 4M, μw, 150 °C , 5 min.

Entry	Ar	Yield[a]
207a	2-ClC_6H_3	66%
207b	C_6H_5	45%
207c	2-CF_3C	42%
207d	2,6-$Cl_2C_6H_3$	66%
208a	2-ClC_6H_3	81%
208b	C_6H_5	76%
208c	2-FC_6H_4	72%
208d	2-EtC_6H_4	81%
208e	1-Np	80%

a) yield reported for step b and step d, Scheme 5

Figure 62. The ^{1}H NMR (DMSO-d_6) spectrum of crude **206** from reaction of **205** with 1 equiv of NBS.

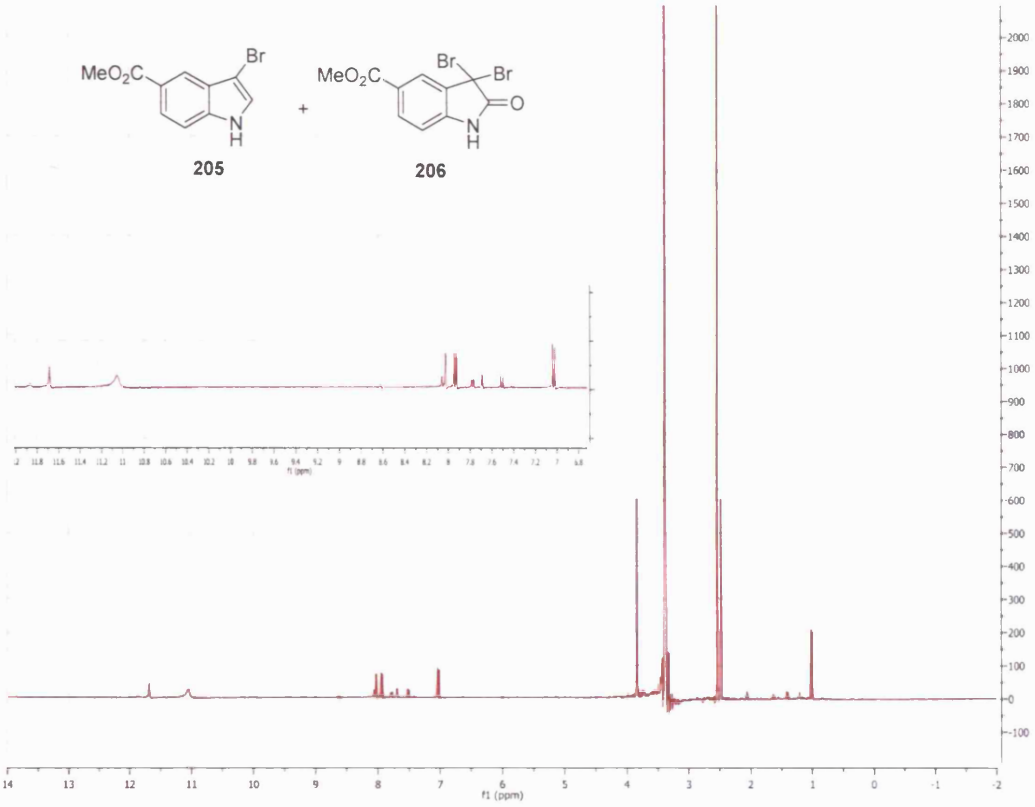
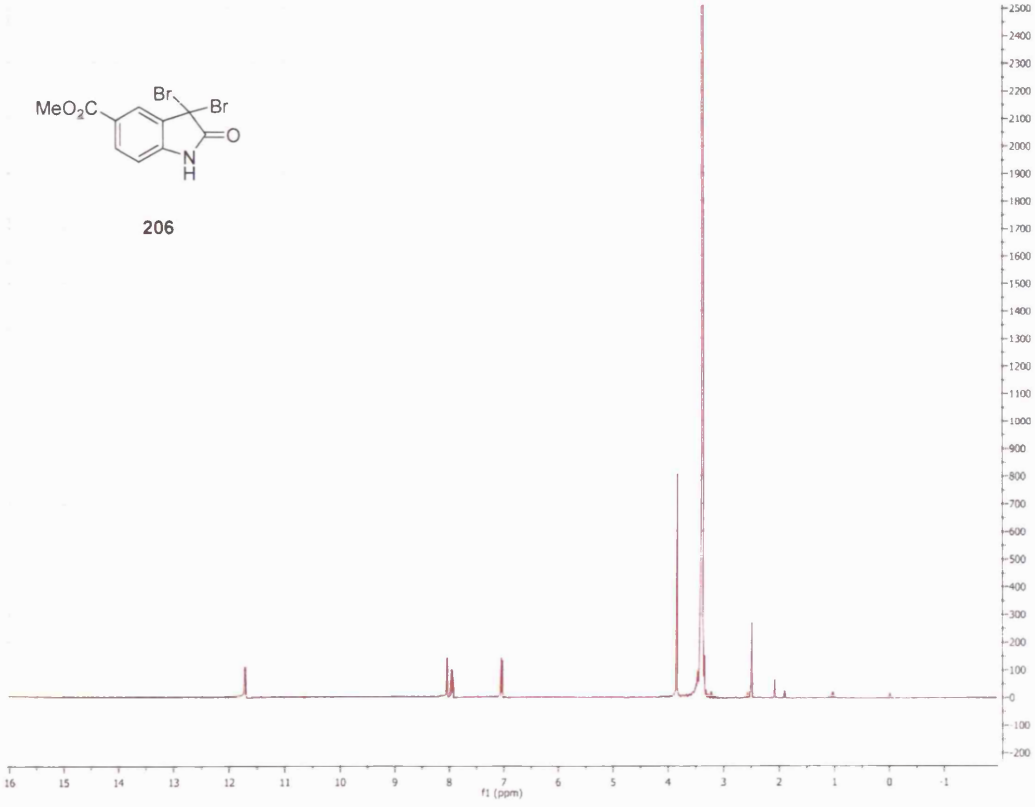


Figure 63. The ^{1}H NMR (DMSO-d_6) spectrum of pure **206** from reaction of **204** with 4 equivalent of NBS.



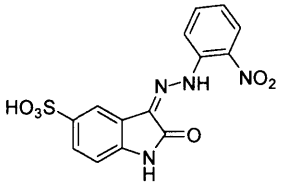
We initially assumed that the mechanism of formation of **207** involved the conversion of **206** into the keto product **203** followed by the condensation with the arylhydrazine. However, compound **207a** was also obtained by reacting **207** and 2-chlorophenylhydrazine in anhydrous methanol. This suggests the possibility that the formation of **207a** might occur via direct displacement of bromine by the hydrazine. Ester **207a** was finally saponified in aqueous NaOH to give acid **208a**. Aqueous hydrochloric acid was found to be effective to promote the hydrolysis of **206** producing the carboxylic acid **209**. Acidic hydrolysis of gem-dibromo derivatives to afford the corresponding keto products has previously described in the literature.[396,397] However, to our best knowledge, this is the first reported example of microwave-assisted hydrolysis. Prompted by the previous successful results, we also wanted to examine the possible development of a one-pot microwave protocol for the synthesis of the series **208**. Unfortunately, reaction of **206** with 2-chlorophenylhydrazine in aqueous hydrochloric acid (microwave heating, 150 °C, 10 min) failed in producing **208a** and pure **207a** was recovered. Presumably, under these conditions, the formation of the hydrazone proceeds faster than the ester hydrolysis causing the precipitation of **207a** from the reaction mixture due to its insolubility in aqueous HCl. Consistently with this hypothesis, when we reacted **207a** with aqueous HCl at 150 °C for 5 min in the microwave, the pure starting material was recovered and no ester hydrolysis was observed. The alternative and versatile procedure we developed for the synthesis of **208** (Table 19) is exemplified by the following reaction. A mixture of **206** (0.149 mmol) in HCl (aq, 4M, 2 ml) was microwave-heated at 150 °C for 5 min. After cooling to room temperature, 2-chlorohydrazine (0.149 mmol) was added to the reaction mixture, which was heated in the microwave at 150 °C for 5 min. After cooling to room temperature, the yellow precipitate was collected by filtration, washed with water (5 ml), cold methanol (2 ml) and dried to afford pure **208a** as a yellow solid (0.121 mmol, 81%) without further purification.

Chapter 9

9.0 Evaluation of oxindole derivatives as SHP-2 inhibitors

9.1 Introduction

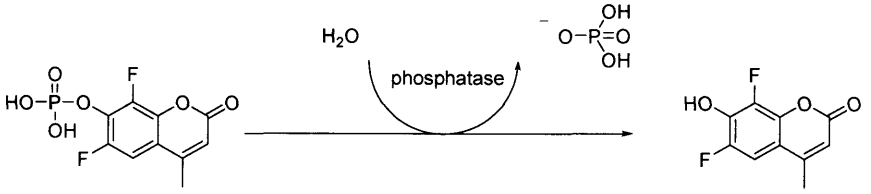
In the course of a medicinal chemistry program aimed at the identification of potential SHP-2 inhibitors targeting the PTP domain of the protein, the oxindole derivative **NSC117199** emerged as a lead compound of moderate potency (IC_{50} of 47 μM) from a high throughput screen of the NCI Diversity set, a library of 1981 compounds.



NSC117199, SHP-2 IC_{50} 47 μM

The screen was conducted using a fluorogenic SHP-2 PTPase assay using a GST fusion protein of SHP-2 PTPase domain (GST-SHP-2PTPase) and 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) as a substrate.[400] The activities of the GST-fusion human recombinant SHP-2PTP, was measured using 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP), a fluorinated MUP derivative developed by Molecular Probes as substrate. In the primary reaction, DiFMUP is transformed into the corresponding fluorogenic hydrolysis product (DiFMU) 6,8-difluoro-4-methylumbelliferone upon dephosphorylation. Therefore, the enzyme activity is associated with in an increase in fluorescence sensitivity. Fluorescence emission from DiFMU is measured at 355/460 nm with a multiwell plate reader (Wallac Victor 1420 multilabel counter, Perkin Elmer Co) (Scheme 22).

Scheme 22. Primary reaction in the DiFMUP assay



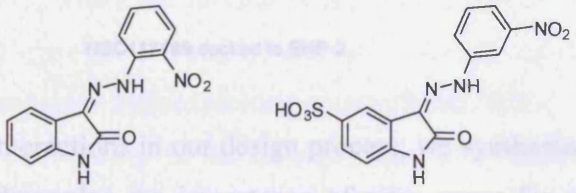
DiFMUP, *non fluorescent* DiFMU, *fluorescent*

We were intrigued by the discovery of the drug-like oxindole scaffold as a new potential template for the design of SHP-2 inhibitors, and by the enormous chemical and structural diversity that could be introduced in the oxindole scaffold in order to optimise the inhibitory activity of the initial lead. Therefore, we synthesized several derivatives of **NSC117199** and initially evaluated them for their ability to inhibit SHP-2 activity using the DiFMUP *in vitro* assay. Here we report our lead optimization directed to exploration of SAR around the oxindole moiety.

9.2 Biological results

All the new synthesized compounds were evaluated in the fluorogenic DiFMUP assay for their ability to inhibit SHP-2 activity. In an effort to improve the potency and determine the structural features responsible for the activity, initial SAR studies were carried out in our laboratories to establish the effects of the sulfonic acid group upon activity. We first examined the removal of the sulfonic acid moiety in compounds **NSC117199** to give **209** (Figure 64).

Figure 64. Structures and *in vitro* SAR of the early oxindole derivatives **209** and **210**.



209, SHP-2 IC_{50} > 100 μM **210**, SHP-2 IC_{50} >100 μM

The loss of activity of derivative **209** revealed the critical role of the sulfonic acid moiety (Figure 64) in capturing important interactions leading to good activity. Moreover, the drop in potency of derivative **210** (IC_{50} >100 μM) revealed that the nitro group at the *ortho*-position of the phenylhydrazone moiety is favourable for optimal activity. The molecular model of **NSC117199** docked to the SHP-2 PTP catalytic pocket suggested hydrogen bonding interactions with the amino acid residues crucial for the Shp-2 catalytic activity (Figure 65 and 66). In fact, the nitro group of **NSC117199** forms hydrogen bond to Arg465, the catalytic nucleophile Cys459, and Ser460 (Figure 66). The sulfonate group forms hydrogen bond to Arg362 and Lys364. The model also reveals a hydrogen bond between the indolinone NH and Asp425, that functions as a general acid in the catalysis of the phosphotyrosine (Figure 66).

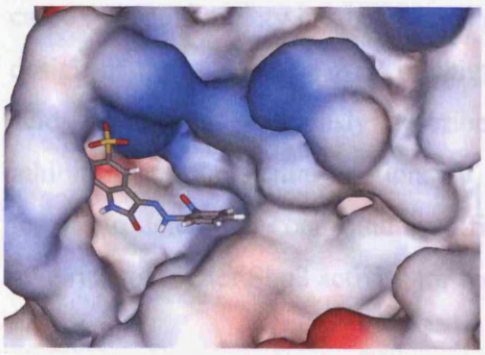
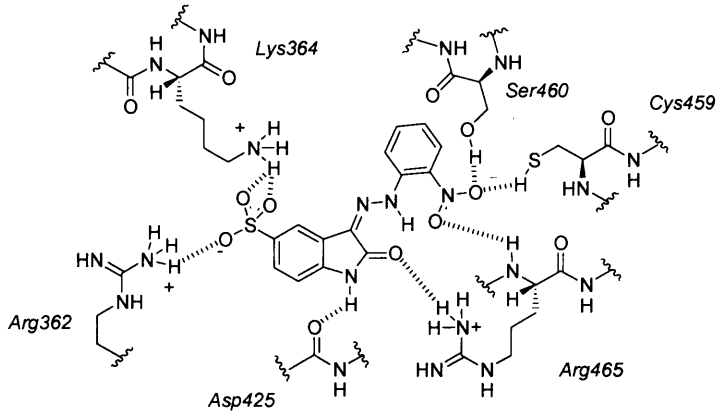


Figure 65. **NSC117199** bound to SHP-2 PTP binding site.

Figure 66. Schematic docking modes of **NSC117199** with SHP-2.



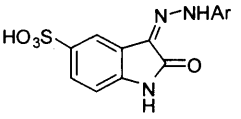
NSC117199 docked to SHP-2

Keeping in mind these interactions in our design process, we synthesized a small library **194** (Table 20) in order to determine the importance of nitro group for activity. As shown by compound **194a**, removal of nitro group resulted in a dramatic loss of potency. We also studied the influence of the nitro group moiety through the incorporation of different substituents at the *ortho*-position of the hydrazone terminus of **NSC117199** (Table 20, entry **194b**, **194d**, **194e**, **194k**). The derivatives bearing a methyl, ethyl, fluorine, trifluoromethyl group, exhibited a significant loss of inhibitory activity. A key component of the lead optimization process was the improvement of the drug-like properties of the original hit, by replacing the nitro group with alternative functionalities that are metabolically stable and capable of capturing and perhaps enhancing the hydrogen bonding interaction to Arg465, Cys459, and Ser460. The nitro group is well known to be easily metabolized in the human body by enzymes generating the corresponding amine, and reactive nitroso derivatives.[401,402] This could lead to loss of activity or, perhaps, to an increased toxicity due to the properties of the metabolites.

The carboxylic acid group was thought to be an appropriate replacement. Furthermore, the carboxylic acid moiety has been previously reported as phosphate mimicking featuring in the design of many selective PTP inhibitors.[248,253-255,258-260] Bis-carboxylic acids or bis-sulfonic acids have been previously described as templates for development of PTP selective inhibitors and the spatial relationship between the two groups appeared to be an important feature to determine good binding affinity.[246,248,262] Unfortunately, this modification did not give rise to a better Shp2 activity for the oxindole derivative **194k** (Table 20).

Table 20. *In vitro* SAR of library **194**.

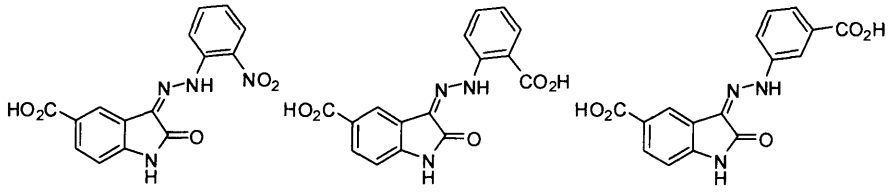
Entry	Ar	SHP-2 IC_{50} (μM)
194a	C_6H_5	>100
194b	2-$CH_3C_6H_4$	>100
194d	2-$CH_2CH_3C_6H_4$	>100
194e	2-FC_6H_4	>100
194f	2-$CF_3C_6H_4$	33-100
194k	2-$CO_2HC_6H_4$	>100



194

Moreover, as emerged from concurrent work in our laboratories, loss in potency was observed in compound **211**, where the sulfonic acid group of **NSC117199** was replaced with the carboxylic acid (Figure 67). Notably, the SO_3H-CO_2H substitution proved to be successful leading to compound **212**, exhibiting a remarkable SHP-2 inhibition (Figure 67). Switching the carboxyl acid moiety to the *meta*-position (**213**) resulted in loss of potency (Figure 67). Although apparently contradictory, the difference in potency between **194k** and **212** may indicate the existence of different binding modes. Conclusions cannot be drawn based on these preliminary results, but a deeper evaluation of this class of compounds is needed in order to understand the mode of action. The X-ray structural determination of compound **212** bound to SHP-2 would be helpful in rationalizing its high potency and our apparently incongruent results.

Figure 67. Structures and SAR of SHP-2 inhibitor **211**, **212**, and **213**.



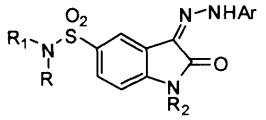
211, SHP-2 IC_{50} >100 μM **212**, SHP-2 IC_{50} 0.8 μM **213**, SHP-2 IC_{50} 15 μM

Next, the library of sulfonamides **191** was prepared to investigate the effect of incorporation of hydrophilic and hydrophobic alkyl and aryl groups (Table 21).

From this set of **NSC117199** derivatives, a significant increase in potency was observed for compounds **191a**$_1$, and **191a**$_{20}$ (IC_{50} 11.9 and 4.4 μM, respectively). The SAR of the *N*-alkylsubstituted sulfonamide series **191** indicated that the introduction of hydrophobic groups such as a methyl (**191a**$_2$), or propyl (**191a**$_7$) group did not give rise to better Shp2 binding affinity. The *N*,*N*-dimethylamino derivative **191a**$_2$ exhibits a drastic drop in potency

compared to compound **191a$_1$**, suggesting the possible engagement of the NH in important key hydrogen bonding interaction. The *N*-iso-propyl sulfonamide **191a$_8$** (IC_{50} 49.6 μM) displayed comparable potency to the parent compound **NSC117199**. However, presumably due to the increased bulk, loss of potency was observed when the isopropyl motif was replaced with the larger *sec*-butyl group to afford compound **191a$_{10}$**. Incorporation of hydrophilic substituents such as *N,N*-dimethylethyl (**191a$_6$**), 2-methoxyethyl (**191a$_9$**), morpholinyl (**191a$_{11}$**), and tetrahydrofurfuryl (**191a$_{12}$**) groups resulted in loss of potency. A series of aromatic sulfonamides were also synthesized. The 4-(aminomethyl)pyridyl (**191a$_{18}$**), 2-(aminomethyl)pyridyl (**191a$_{19}$**), furfuryl (**191a$_{13}$**), and 2-thiophenemethyl (**191a$_{14}$**) derivatives were found to be inactive. Enhanced potency was observed for the 4-chlorobenzylsulfonamide **191a$_{20}$** (IC_{50} 10.12 μM). The *para* Cl→OMe substitution was also studied (**191a$_{16}$**), but resulting in loss of potency. Switching the methoxy group from the 4- to the 3-position (**191a$_{15}$**) did not give rise to better SHP-2 binding affinity. These findings generally indicated a tendency of the binding potency to increase concomitantly with the hydrophobicity. Loss in potency was also observed for the benzylsulfonamide (**191a$_{36}$**). The size and hydrophobicity of the chlorine appears be pivotal, gaining favourable van der Waals interaction with hydrophobic residues in the PTP binding site. Moreover, *N*-methyl sulfonamide **191a$_{17}$** was not active, possibly suggesting that the sulfonamide NH may form hydrogen bond interactions critical for the protein/ligand affinity. However, the synthesis of a N-(4-chlorobenzyl)-N-methyl sulfonamide derivative would be necessary to verify the hypothesis. Additional studies to expand the SAR information are currently ongoing in our laboratories. Synthetic efforts are directed toward the synthesis of novel compounds containing various hydrophobic substituents at the 2-, 3-, and 4-positions of the benzyl mioety at the sulfonamide terminus of **191a$_{20}$**.

Table 21. *In vitro* SAR for the sulfonamides **191**.



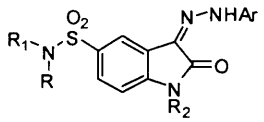
191

Entry	R	R_1	R_2	Ar	SHP-2 IC_{50} (μM)
191a$_1$	H	H	H	2-$NO_2C_6H_4$	11.9
191a$_2$	Me	Me	H	2-$NO_2C_6H_4$	33-100
191a$_3$	Me	Me	Me	2-$NO_2C_6H_4$	>100
191a$_4$	Me	Me	Et	2-$NO_2C_6H_4$	>100
191a$_5$	Me	Me	Benzyl	2-$NO_2C_6H_4$	>100
191a$_6$	H	*N,N*-dimethylethyl	H	2-$NO_2C_6H_4$	>100
191a$_7$	H	Propyl	H	2-$NO_2C_6H_4$	>100
191a$_8$	H	*Iso*-propyl	H	2-$NO_2C_6H_4$	49.6
191a$_9$	H	2-Methoxyethyl	H	2-$NO_2C_6H_4$	>100
191a$_{10}$	H	*Sec*-butyl	H	2-$NO_2C_6H_4$	>100
191a$_{11}$	H	Morpholinyl	H	2-$NO_2C_6H_4$	>100
191a$_{12}$	H	Tetrahydrofurfuryl	H	2-$NO_2C_6H_4$	>100
191a$_{13}$	H	Furfuryl	H	2-$NO_2C_6H_4$	>100
191a$_{14}$	H	2-Thiophenemethyl	H	2-$NO_2C_6H_4$	>100
191a$_{15}$	H	3-Methoxybenzyl	H	2-$NO_2C_6H_4$	>100
191a$_{16}$	H	4-Methoxybenzyl	H	2-$NO_2C_6H_4$	>100
191a$_{17}$	Me	Benzyl	H	2-$NO_2C_6H_4$	>100
191a$_{18}$	H	4-(Aminomethyl)pyridyl	H	2-$NO_2C_6H_4$	>100
191a$_{19}$	H	2-(Aminomethyl)pyridyl	H	2-$NO_2C_6H_4$	33-100
191a$_{20}$	H	4-Chlorobenzyl	H	2-$NO_2C_6H_4$	4.4
191a$_{36}$	H	Benzyl	H	2-$NO_2C_6H_4$	70% inhibition at 100 μM

With the SAR of the sulfonamide portion established, the subsequent lead optimization was directed to the enhancing the observed activities of the new leads **191a$_1$**, **191a$_{20}$**, and **191a$_8$** (Table 22). IC_{50} values were systematically determined only for compounds that inhibit 100% of SHP-2 phosphatase activity at 100 μM. Results from concurrent work in our labs, showed that replacement of the nitro group in compound **191a$_1$**, **191a$_{20}$**, and **191a$_8$** with chlorine resulted in loss of inhibitory potency (data not shown).

We first systematically evaluated the position of the nitro group on the phenylhydrazone moiety for compounds **191a$_1$** and **191a$_{20}$**. The analogues **191a$_{32}$**, **191a$_{34}$**, **191a$_{33}$**, and **191a$_{35}$** displayed a lower potency compared to the parent compounds, suggesting that the carboxylic acid in the *meta* position seems in general to be the favourable pattern (Table 22).

Table 22. *In vitro* SAR for the sulfonamides **191**.



191

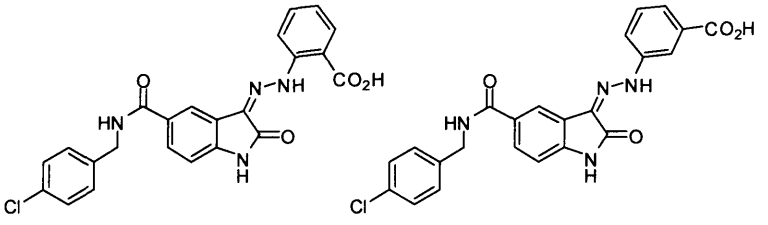
Entry	R	R_1	R_2	Ar	SHP-2 IC_{50} (μM)
191a$_{21}$	H	*Iso*-propyl	H	C_6H_5	>100
191a$_{23}$	H	*Iso*-propyl	H	2-$CO_2HC_6H_4$	7.94
191a$_{26}$	H	*Iso*-propyl	H	3-$CO_2HC_6H_4$	4.5
191a$_{29}$	H	*Iso*-propyl	H	4-$CO_2HC_6H_4$	4.5
191a$_{32}$	H	H	H	3-$NO_2C_6H_4$	63% inhibition at 100 μM
191a$_{34}$	H	H	H	4-$NO_2C_6H_4$	28% inhibition at 100 μM
191a$_{24}$	H	H	H	2-$CO_2HC_6H_4$	19.7
191a$_{27}$	H	H	H	3-$CO_2HC_6H_4$	12.4
191a$_{30}$	H	H	H	4-$CO_2HC_6H_4$	27.5
191a$_{33}$	H	4-Chlorobenzyl	H	3-$NO_2C_6H_4$	48% inhibition at 100 μM
191a$_{35}$	H	4-Chlorobenzyl	H	4-$NO_2C_6H_4$	53% inhibition at 100 μM
191a$_{25}$	H	4-Chlorobenzyl	H	2-$CO_2HC_6H_4$	23.7
191a$_{28}$	H	4-Chlorobenzyl	H	3-$CO_2HC_6H_4$	1.0
191a$_{31}$	H	4-Chlorobenzyl	H	4-$CO_2HC_6H_4$	15.4

Replacement of the nitro group with the carboxylic acid group was also studied. Analogues **191a$_{24}$**, **191a$_{27}$**, and **191a$_{30}$** displayed a lower potency compared to the parent compounds **191a$_1$**. (IC_{50} values of 19.7, 12.4, and 27.5 μM, respectively). Finally, from the screening of the second generation library, compound **191a$_{28}$** emerged as a potent SHP-2 inhibitor (IC_{50} 1.0 μM). Substitution at the meta-position appeared to be optimal for good activity as shown by the significant low potency of the *ortho* and *para*-carboxylsulfonamides **191a$_{25}$** and **191a$_{31}$** (IC_{50} values of 23.7, 15.4 μM, respectively). The $NO_2 \rightarrow CO_2H$ substitution proved to be successful for compound **191a$_8$**. The new analogue **191a$_{23}$**, and its positioned isomers **191a$_{26}$** and **191a$_{29}$**, exhibited much improved binding affinity (IC_{50} 7.94, 4.5, and 4.5 μM, respectively). In addition, the analogue **191a$_{21}$** with an unsubstituted arylgroup was not active. The requirement of the carboxylate may be due to its ability to capture important hydrogen-bonding interaction as well as acting more generally as a phosphate mimic.

Other work in our laboratories also suggests that the sulfonamide functionality is important for phosphatase inhibitory activity. As shown in Figure 68, when compared to their sulfonamides counterparts, the analogous amides show reduced activity. This is probably due to the different conformational properties of amide bond (Figure 68). Distinct differences are the lower rotation barriers around the SN bond, and the tetrahedral geometry of the sulfonamide group in comparison to the planar arrangement of the amide bond, thus making the sulfonamides more flexible. These conformational properties lead to different hydrogen

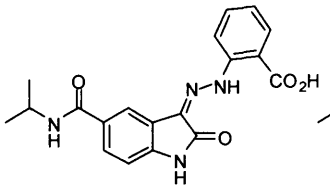
bonding behaviour. For instance, the N-H and C=O of a secondary carboxamide cannot interact simultaneously with a closely acceptor/donor pair. In contrast, the N-H and S=O of a secondary sulfonamide can achieve this type of interaction. The introduction of sulfonamide group increases polarity of a molecule and the hydrogen bond donor properties as a sulfonamide N-H is more acidic (Pk_a 11-12) than a carboxamide (Pk_a 15-16).[403,404] Moreover, the *meta*-substitution pattern at the phenylhydrazone moiety appeared to be favourable for good activity, as displayed by the enhanced potency of derivative **215** compared to the isomeric **214**.

Figure 68. Structures and SAR of carboxamides **214-217**.

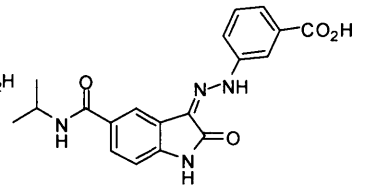


214, SHP-2 IC_{50} > 100 μM

215, SHP-2 IC_{50} > 32.2 μM



216, SHP-2 IC_{50} > 100 μM

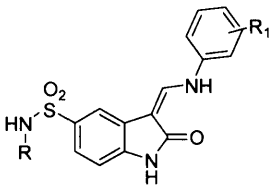


217, SHP-2 IC_{50} > 100 μM

Loss of SHP-2 inhibitory activity was also observed by replacing the hydrazone linker with a N-substituted exocyclic methylene at the 3-position (**199c**, **199e**, **200a**, **200b**, **200c** IC_{50} > 100 μM), respectively (Table 23). This may suggest the involvement of the possible engagement of the N in important key interactions and the requirement of a hydrophilic linker for optimal activity. Replacement of the hydrazone linkage with with an enamine has been described in recent literature. A group from GlaxoSmithKline described a novel class of CDK2 oxindole-based inhibitors containing hydrazone and enamine connection.[394] The replacement of the hydrazone linkage with an enamine strategy resulted inconsequential to enzyme binding, providing access to expanded diversity on phenyl ring at the enamine-terminus.

Table 23. *In vitro* SAR of library **199** and **200**.

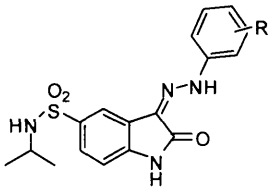
Entry	R	R_1	SHP-2 IC_{50} (μM)
200a	*Iso*-propyl	2-CO_2H	33-100
200b	*Iso*-propyl	3-CO_2H	33-100
200c	*Iso*-propyl	4-CO_2H	33-100
199d	*Iso*-propyl	2-NO_2	>100
199e	4-Chlorobenzyl	2-NO_2	>100



199-200

We also prepared a small library of primary amides **193**, further developing compounds **191a$_{23}$** and **199a$_{26}$**, and further probing the role of the carboxylic acid (Table 24). The newly synthesized compounds displayed a dramatic loss of potency regardless the hydrophobicity, hydrophilicity, or size of the groups we introduced. Steric factors may play a key role in accommodating the larger substituents (e.g. furfuryl, benzyl, 2-(aminomethyl)pyridyl, 2-morpholin-4-yl-ethyl) inside the active site. However, not even small groups such as hydrogen were tolerated, further indicating that the carboxylic acid may interact with the PTP catalytic site acting as a mimic of the phosphate group in pTyr.

Table 24. *In vitro* SAR of library **193**.



193a-i, R = 2-CONHR'
193j-q, R = 3-CONHR'

Entry	R = 2-CONHR$_1$, R$_1$ =	SHP2 IC_{50} (μM)	Entry	R = 3-CONHR$_1$, R$_1$ =	SHP2 IC_{50} (μM)
193a	Furfuryl	33-100	**193j**	Furfuryl	33-100
193b	H	>100	**193k**	H	33-100
193c	Dimethylaminoethyl	>100	**193l**	Dimethylaminoethyl	activator
193d	2-Methoxyethyl	>100	**193m**	2-Methoxyethyl	42.3
193e	Benzyl	>100	**193n**	Benzyl	>100
193f	2-(Aminomethyl)pyridyl	33-100	**193o**	2-(Aminomethyl)pyridyl	25.0
193g	2-Morpholin-4-yl-ethyl	>100	**193o**	2-Morpholin-4-yl-ethyl	>100
193h	Me	>100	**193p**	Me	>100
193i	Et	>100	**193q**	Et	>100

9.3 Selectivity

A major goal of our study was to improve the SHP-2 activity of the lead compound **NSC117199**, as well as SHP-2/SHP-1 selectivity. For the specificity test, we have also performed *in vitro* assay and examined the effect of the activity on phosphatase PTP1B

(Protein Phosphatase Receptor 1B). As summarized in Table 25, compound **191a$_1$** and **191a$_{28}$** exhibited good selectivity for SHP-2 (approximately 9- and 14-fold, respectively). In all cases the compounds showed greater inhibitory properties for SHP-2 when compared to PTP1B.

Table 25. In vitro selectivity of compounds **191a$_1$**, **191a$_{20}$**, **191a$_{27}$**, and **191a$_{28}$**.

Entry	SHP-2 IC_{50} (μM)	SHP-1 IC_{50} (μM)	PTP1B IC_{50} (μM)
191a$_1$	11.9	103.7	156.6
191a$_{20}$	4.4	40.9	9.8
191a$_{27}$	4.5	15.7	37.1
191a$_{28}$	1.0	14.2	4.4

In an effort to rationalize the great selectivity, sulfonamide **191a$_1$** was docked within the PTP-binding cleft of SHP-2 and SHP-1. For docking studies we employed the X-ray crystal structure of full length SHP-2[223] (pdb 2SHP) determined at 2.0 Å resolution and the X-ray crystal structure of catalytic domain PTP1c of SHP-1[405] (pdb 1fpr) determined at 2.0 Å. For SHP-2, the N-SH2 domain was removed and then the 3D molecular model of **191a$_1$** was docked by GLIDE[406] into the PTP binding site.

As shown in Figure 69 and 70, the model revealed distinct binding modes of **191a$_1$** to SHP-2 and SHP-1. For SHP2, compound **191a$_1$** is bound deep inside in the pocket. Two hydrogen bonds were formed between the sulfonamide functionality and SHP-2. Specifically, the sulfonamide oxygen was hydrogen-bonded with the backbone NH of Gly427 and the NH terminal of Gln510 (2.43 and 1.93 Å, respectively). The model also reveals that the orientation of the ligand allows the formation of a hydrogen bond between the NH of the hydrazone linker OH of Ser460 (2.22 Å). Hydrogen bonds are also bridging the nitro O-atom and the oxindole O-atom to the OH of Ser460 (1.98 and 1.95 Å, respectively). The strong interaction with Ser460 are presumably contributing towards the SHP-2 binding affinity of **191a$_1$** and the SHP-2/SHP-1 selectivity. When docked to SHP-1 (Figure 69B and 70B), compound **191a$_1$** displayed a weaker binding affinity (-4.26 kcal) in its lowest energy pose. This may explain its selectivity toward SHP-2. For SHP-1, the docked structure of **191a$_1$** reveals that the *o*-nitrophenyl hydrazone functionality is buried inside the pocket, leaving the NH and the carbonyl of oxindole, and the O-atoms of the sulfonamide pointing out exposed to the solvent area (Figure). The ligand makes contact to the enzyme surface through a hydrogen bond interaction between the nitro O-atom and Ala457 (2.95 Å). The NH_2 of the sulfonamide hydrogen bonds Asp421. The two different binding modes of **191a$_1$** may explain the greater SHP-2 binding affinity.

Figure 69. Schematic docking modes of **191a$_1$** with SHP-2 (A) and SHP-1(B).

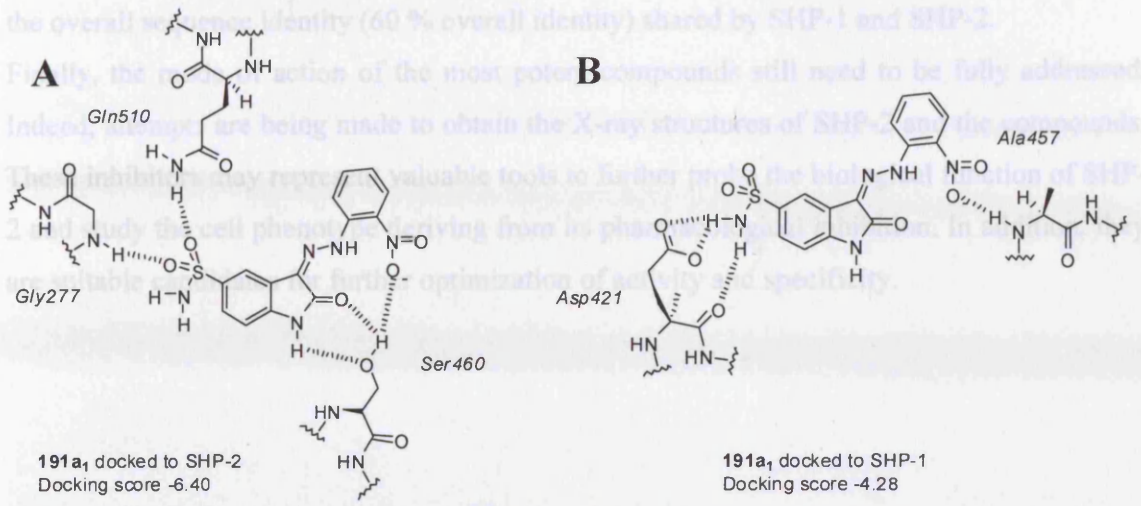
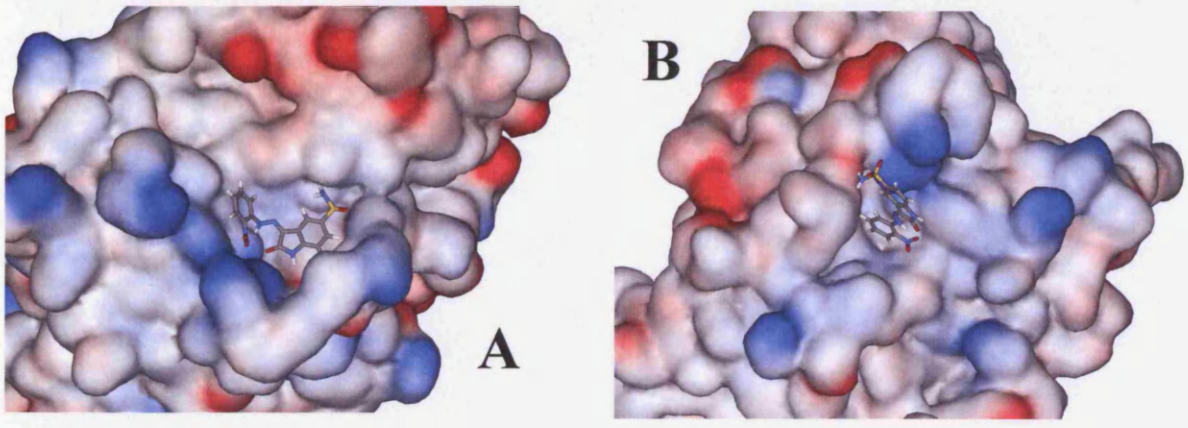


Figure 70. (A) **191a$_1$** bound to SHP-2 PTP binding site. (B) **191a$_1$** bound to SHP-1 PTP binding site. The protein surface of the enzymes is colored according to atomic charges. Positively charged areas are colored in blue and negatively charged areas are colored in red.



9.4 Conclusion

In the search of novel SHP-2 inhibitors, the oxindole derivative **NSC117199** emerged as a lead compound from a high throughtput screen of the NCI Diversity set. SAR studies around the oxindole scaffold led us to determine some of the structural features of **NSC117199** that are responsible for the activity. Ultimately new more potent SHP-2 inhibitors were discovered also indicating the versatility of the oxindole scaffolds as a valid template for development of SHP-2 inhibitors. This has resulted in a 47-fold increase in activity against SHP-2. In addition high selectivity between SHP-1 and SHP-2 was observed for **191a$_1$**, and

191a$_{28}$ indicating that development of SHP-2 selective inhibitors could be achieved despite the overall sequence identity (60 % overall identity) shared by SHP-1 and SHP-2.

Finally, the mode of action of the most potent compounds still need to be fully addressed. Indeed, attempts are being made to obtain the X-ray structures of SHP-2 and the compounds. These inhibitors may represent valuable tools to further probe the biological function of SHP-2 and study the cell phenotype deriving from its pharmacological inhibition. In addition, they are suitable candidates for further optimization of activity and specificity.

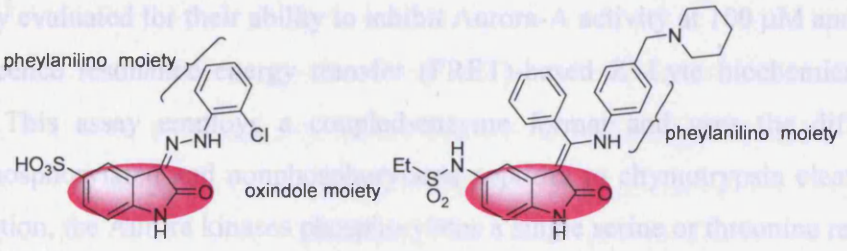
Chapter 10

10.0 Evaluation of oxindole derivatives as Aurora kinases inhibitors

10.1 Introduction

As part of our program toward the development of Aurora kinases inhibitors, the oxindole derivative **HL10581** emerged as a lead compound from a high throughput screen for Aurora-A (Figure 72). The oxindole moiety is a drug-like scaffold that has also featured in the design of Cyclin-Dependent kinase 2 (CDK2) kinase, and Caspase 3 and 7 inhibitors.[392,394] Hesperadin (**79**), that shares an oxindole scaffold and the phenylanilino moiety, has been reported as a potent inhibitor of Aurora-B[294] (Figure 72). The lead optimization, directed to the exploration of SAR around the oxindole scaffold, led us to the identification of potent *in vitro* inhibitors of Aurora-A and -B, showing nanomolar potency.

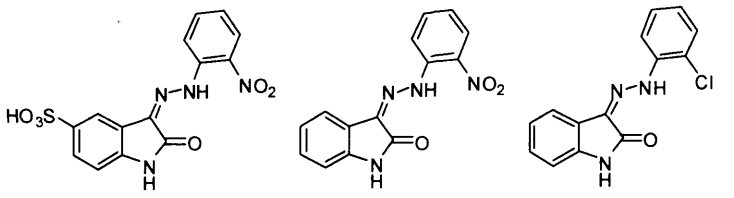
Figure 72. The initial hit **HL10581** identified from the screening compound library and Hesperadin (**2**). Common features are highlighted in pink.



10.2 Biological results

After preliminary modification of derivative **HL10581**, compound **219**, exhibiting 50% inhibition of Aurora-A at 10 μM, was also identified as a hit for further investigation (Figure 73). Moreover, in an effort to determine the structural features responsible for the activity, initial SAR studies were carried out to establish the requirements of the sulfonic acid group motif for optimal activity. We first examined the removal of the sulfonic acid moiety from compounds **HL10581** and **HL1056 219** to give **220** and **209**, respectively. The weak activity of derivatives **219** and **209** revealed the critical role of the sulfonic acid moiety (Figure 73).

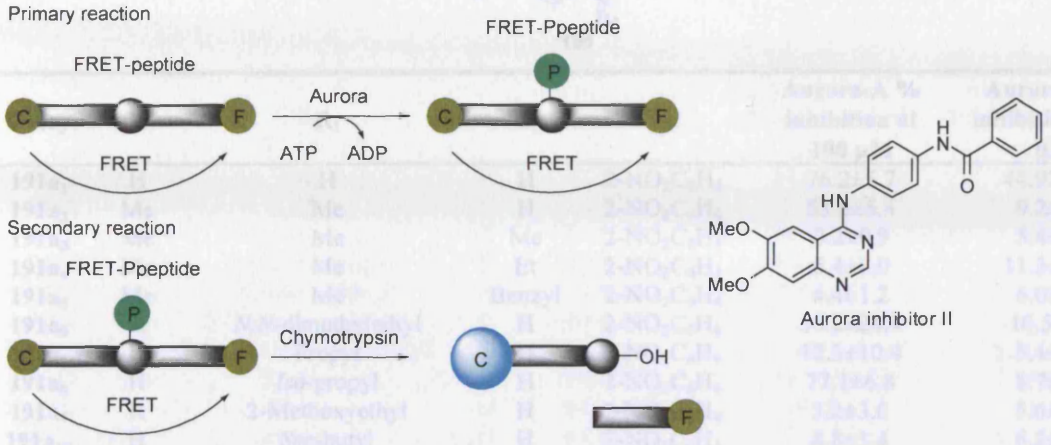
Figure 73. Structure and *in vitro* SAR for the early key oxindole derivatives analogues of **HL10581**. The values were determined at 10 μM concentration.



We began our work by preparing the library of sulfonamides **191** (Table 26) aimed at further probing the role of the free sulfonic acid group, and the effect of incorporation of hydrophilic and hydrophobic alkyl and aryl groups at the 5-postion of **HL10581** and **219** (Figure 72 and 73). The library of sulfonamides **221** was previously synthesized in our laboratories. Here we report the biological results from both series **191** and **221**. All the synthesized compounds were preliminary evaluated for their ability to inhibit Aurora-A activity at 100 μM and 10 μM using a fluorescence resonance energy transfer (FRET)-based Z'-Lyte biochemical assay (Figure 74).[407] This assay employs a coupled-enzyme format and uses the differential sensitivity of phosphorylated and nonphosphorylated peptides to chymotrypsin cleavage. In the primary reaction, the Aurora kinases phosphorylates a single serine or threonine residue in a synthetic FRET-peptide. This FRET-peptide is doubly labeled with a fluorophore at each end—coumarin (the FRET donor) on one end and fluorescein (the FRET acceptor) on the other—and also contains a single phosphorylation site which either overlaps with or lies adjacent to the proteolytic site. In the secondary reaction, a site-specific protease recognizes and cleaves the phosphorylated FRET-peptide. Cleavage disrupts FRET between the donor and acceptor fluorophores on the FRET-peptide, whereas uncleaved, phosphorylated FRET-peptides maintain FRET. Aurora kinase non-phosphorylated FRET-peptides cannot be cleaved by the chymotrypsin protease. Upon excitation of the donor fluorophore (coumarin) due to FRET, the uncleaved FRET-peptide yields a coumarin fluorescence signal (at 445 nm) and a fluorescein fluorescence signal (at 520 nm). Cleavage disrupts FRET and causes a decrease in the fluorescein fluorescence signal and a strong increase in the coumarin fluorescence signal. Therefore, the assay uses a ratiometric method, which calculates the ratio of donor emission to acceptor emission (the emission ratio) after excitation of the donor fluorophore at 400 nm, to quantitate reaction progress (Figure 74). The recombinant Aurora-A was incubated with synthetic FRET-peptide substrate in a kinase buffer containing 100 μM or 10 μM the compounds. Aurora inhibitor II (4-(4'-benzamidoanilino)6,7-

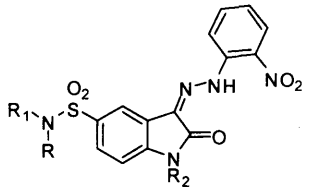
dimethoxyquinazoline) was used as positive control (Figure 74) (IC_{50} 310 nM and 240 nM for Aurora A and B, respectively).

Figure 74. Schematic diagram of the (FRET)-based Z'-Lyte biochemical assay and the Aurora inhibitor II.



IC_{50} values were systematically determined only for compounds that inhibit >85% of Aurora-A kinase activity at 10 μM. Unfortunately, all the member of the library exhibited a dramatic decrease in potency. As shown in Table 26 and 27, at 10 μM concentration, the % inhibition generally ranges between 5 and 11%, while compounds **191a$_1$** and **221k** respectively inhibit 45 and 31% of Aurora-A kinase activity. Not even the introduction of small groups such as hydrogen (**191a$_1$** and **221b**) and methyl (**191a$_2$** and **221a**) was tolerated. These findings clearly suggested the important role of the sulfonic acid moiety for good activity. Moreover, sulfonamides **191** and **221** may also assume different conformation leading to different binding modes, where the ligand/enzyme interactions are weaker resulting in loss of potency. Notably, compounds **191a$_1$**, **191a$_8$**, **221b**, approximately inhibit 76-79% of Aurora-A kinase activity at 100 μM concentration.

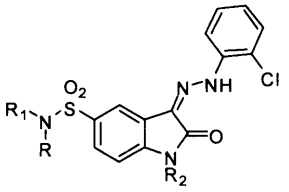
Table 26. *In vitro* SAR for the sulfonamides **191**.



191

Entry	R	R_1	R_2	Ar	Aurora-A % inhibition at 100 μM	Aurora-A % inhibition at 10 μM
191a$_1$	H	H	H	2-$NO_2C_6H_4$	76.2±5.7	44.92±3.5
191a$_2$	Me	Me	H	2-$NO_2C_6H_4$	53.5±5.4	9.2±2.6
191a$_3$	Me	Me	Me	2-$NO_2C_6H_4$	0.2±0.9	5.4±4.0
191a$_4$	Me	Me	Et	2-$NO_2C_6H_4$	6.4±6.0	11.3±10.2
191a$_5$	Me	Me	Benzyl	2-$NO_2C_6H_4$	4.4±1.2	6.0±2.6
191a$_6$	H	*N,N*-dimethylethyl	H	2-$NO_2C_6H_4$	36.2±24.4	10.5±2.6
191a$_7$	H	Propyl	H	2-$NO_2C_6H_4$	12.3±10.4	5.4±1.9
191a$_8$	H	*Iso*-propyl	H	2-$NO_2C_6H_4$	77.1±6.8	8.7±2.7
191a$_9$	H	2-Methoxyethyl	H	2-$NO_2C_6H_4$	3.2±3.0	5.6±0.9
191a$_{10}$	H	*Sec*-butyl	H	2-$NO_2C_6H_4$	8.8±1.4	6.2±0.8
191a$_{11}$	H	Morpholinyl	H	2-$NO_2C_6H_4$	9.6±5.2	7.1±2.7
191a$_{12}$	H	Tetrahydrofurfuryl	H	2-$NO_2C_6H_4$	4.1±1.4	5.1±3.2
191a$_{13}$	H	Furfuryl	H	2-$NO_2C_6H_4$	45.3±10.1	8.7±5.5
191a$_{14}$	H	2-Thiophenemethyl	H	2-$NO_2C_6H_4$	31.1±6.5	7.7±6.1
191a$_{15}$	H	3-Methoxybenzyl	H	2-$NO_2C_6H_4$	5.86±1.9	7.1±1.9
191a$_{16}$	H	4-Methoxybenzyl	H	2-$NO_2C_6H_4$	5.60±2.76	6.11±2.6
191a$_{17}$	Me	Benzyl	H	2-$NO_2C_6H_4$	3.8±2.5	6.3±3.8
191a$_{18}$	H	4-(Aminomethyl)pyridyl	H	2-$NO_2C_6H_4$	24.8±8.4	3.6±2.2
191a$_{19}$	H	2-(Aminomethyl)pyridyl	H	2-$NO_2C_6H_4$	5.7±2.9	5.1±2.4
191a$_{20}$	H	4-Chlorobenzyl	H	2-$NO_2C_6H_4$	24.5±5.8	7.1±3.0

Table 27. *In vitro* SAR for the sulfonamides **221**.



221

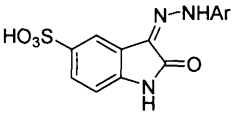
Entry	R	R_1	R_2	Ar	Aurora-A % inhibition at 100 μM	Aurora-A % inhibition at 10 μM
221a	Me	Me	H	2-ClC_6H_4	10.0±3.7	2.8±3.1
221b	H	H	H	2-ClC_6H_4	79.2±10.1	10.7±5.9
221c	H	4-Methoxybenzyl	H	2-ClC_6H_4	22.9±6.3	7.6±2.2
221d	H	4-Chlorobenzyl	H	2-ClC_6H_4	9.7±11.5	5.4±2.0
221e	H	3-Methoxybenzyl	H	2-ClC_6H_4	7.5±4.3	7.1±0.5
221f	H	2-Thiophenemethyl	H	2-ClC_6H_4	13.7±3.4	6.0±3.5
221g	H	2-(Aminomethyl)pyridyl	H	2-ClC_6H_4	9.5±11.6	5.3±1.3
221h	H	Furfuryl	H	2-ClC_6H_4	23.3±5.2	7.5±3.3
221i	H	Propyl	H	2-ClC_6H_4	1.9±4.2	3.3±1.7
221j	H	*Iso*-propyl	H	2-ClC_6H_4	17.5±2.5	6.5±3.6
221k	H	Furfuryl	H	2-ClC_6H_4	57.3±22.5	31.2±19.1
221l	H	2-Methoxyethylyn	H	2-ClC_6H_4	5.4±6.1	5.2±1.8
221m	H	*Sec*-butyl	H	2-ClC_6H_4	4.8±3.7	5.9±2.2
221n	H	Tetrahydrofurfuryl	H	2-ClC_6H_4	12.9±13.5	4.24±0.27
221o	H	4-(Aminomethyl)pyridyl	H	2-ClC_6H_4	44.5±7.2	-2.4±3.3
221p	Me	Benzyl	H	2-ClC_6H_4	8.4±4.8	6.6±2.0
221q	H	3-(Aminomethyl)pyridyl	H	2-ClC_6H_4	5.7±43.3	8.8±4.9
221r	H	*N,N*-dimethylethyl	H	2-ClC_6H_4	17.2±7.4	11.3±2.1

The molecular model of **HL10581** binding to the Aurora-A ATP binding pocket suggested that introduction of hydrophobic and reasonably small substituents into the phenyl ring of the phenylhydrazone moiety could be well tolerated, and thereby increase the binding affinity. Based on the preliminary SAR and the docking studies, we synthesized a focused library of further oxindole derivatives **194** (Table 28). The major goals of the modifications were not only to increase the activity, but also to provide inhibitors with adequate cell permeability and high potency in cellular assays. Derivatives **194** were initially evaluated for their ability to inhibit Aurora-A activity. IC_{50} values were determined only for compounds that inhibit >85% of Aurora-A kinase activity at 10 μM (Table 28). As predicted by the model, hydrophobic and relatively small group were well tolerated. In accordance, replacement of nitro and chlorine with the carboxylic acid motif resulted in a significant drop in potency (**194k**, IC_{50} >100 μM). Among several aromatic rings, the 2-chlorophenyl cannot be considered critical for the activity. In fact, removal of the chlorine resulted in the analogue **194a** (IC_{50} 2.5 μM) of potency comparable to the parent compound **HL10581**. As summarized in Table 28, several other replacements at the *ortho*-position were synthesized. Compound **194b**, **194d**,

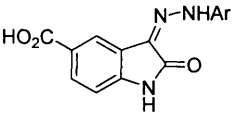
and **194e** were found to maintain the same inhibitory activity, as well as the dichloro derivatives **194c**, **194f**, **194j**. It is well known that, size-wise, fluorine is a good hydrogen mimic adding only limited steric demand at the enzyme site.[408] Fluorination can also aid hydrophobic interaction between the drug and the binding site on the enzyme. A comparison of substituent effect revealed that replacement of the methyl by a trifluoromethyl group at the 2-position resulted in a significant reduction in potency (**194b** versus **194f**). A dramatic difference in activity also exists between the pentafluorophenyl derivative (**194g**) and compound **194a**. To our delight, substitution with naphthyl group caused a significant increase in activity, yielding compound **194h** as potent inhibitor of Aurora-A (IC_{50} 0.540 μM).

Table 28. *In vitro* SAR for the early key oxindole derivatives **194** and **208** analogues of **HL10581**.

Entry	Ar	Aurora-A IC_{50} (μM)
194h	1-Np	0.540
194a	C_6H_5	2.5
194b	2-$CH_3C_6H_4$	3
194d	2-$CH_3CH_2C_6H_4$	7
194e	2-FC_6H_4	7
194f	2-$CF_3C_6H_4$	35
194c	2,6-$Cl_2C_6H_3$	8
194i	2,4-$Cl_2C_6H_3$	5
194j	2,5-$Cl_2C_6H_3$	3.6
194g	C_6F_5	100
194k	2-$CO_2HC_6H_4$	>100
208e	1-Np	1
208b	C_6H_5	10
208d[a]	2-$CH_3CH_2C_6H_4$	
208c[a]	2-FC_6H_4	
208a[a]	2-ClC_6H_4	



194

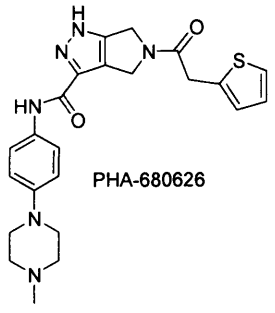


208

a) IC_{50} values were determined only for compounds that inhibit >85% of Aurora-A kinase activity at 10 μM.

Further screening revealed that **194h** inhibits Aurora-B (IC_{50} 0.349 μM) activity *in vitro*. The sulfonic acid was also replaced with the carboxylic acid in the series of analogues **208**. As shown in Table 27, the presence of the sulfonic acid is still critical for good activity and its replacement with the carboxylic acid results in a significant drop in potency. Modest inhibitory activity in the micromolar range was only observed for compound **208a**, bearing a naphthyl at the hydrazone terminus. Carboxylic acids (R-CO_2H) are isosteres of sulfonic acids and share many properties in common, including the ability to act as hydrogen-bond acceptor. However, due to the presence of an additional oxygen, sulfonic acids are more acidic than carboxylic acids. This enhanced acidity results in an increased ionization at

physiological pH and an increased H_2O solubility, which may explain the greater potency of compounds **194h** and **194a** compared to **208e** and **208b**, respectively. However, the modest activity displayed by compound **208d**, also highlights the significant contribution of the hydrophobic Van Der Waals interaction on binding.



The hydrazone **208h** was docked within the ATP-binding pocket of Aurora-A and Aurora-B (Figure 3). For docking studies we employed the X-ray crystal structure of human Aurora-A with ADP bound (pdb 1mq4) determined at 1.90 Å resolution[409] and the X-ray crystal structure of Aurora-B with PHA-680626 (pdb 2j4z) bound determined at 2.00 Å resolution.[306] The ADP and PHA-680626 were removed and then the 3D molecular model of **194h** was docked by GLIDE[406] into the ATP binding site. For Aurora-A, the docked structure of **194h** (Figure 75A and 76) reveals that the naphthyl group occupies the purine base hydrophobic pocket created by residues Ala273, Leu194, Val147, Leu263, Ile139, Ala160, and Ala213 (Figure 77). The sulfonyl group is pointing out of the site forming a hydrogen bond to the Lys141 (Figure 76). The model also reveals a hydrogen bond between the indolinone NH and Asp246. This interaction was considered optimal for activity.

For Aurora-B, the docked structure of **194h** reveals that the sulfonyl group is in the phosphate binding region, leaving the naphthyl group pointing out (Figures 75B and 76). As shown in Figure 77, hydrogen bonds with Ala213, Ile184, and Glu211 are key interactions presumably contributing towards the Aurora-B binding affinity of **194h**. In addition the model reveals an important stabilization via a salt bridge between the sulfonate group and the imidazole group of His280. For the specificity test, we have performed *in vitro* assay and examined the effect on the activity of several serine/threonine kinases, such as PKA, SGK, and ROCK1. The results indicated that **194h** selectively inhibits Aurora-A and –B kinases. Moreover, **rpm223** did not exhibit inhibitory activity for SHP-2.

Figure 75. Molecular model of **194h** binding to the Aurora-A (**A**) and –B (**B**) ATP binding pocket. The protein surface of the enzymes is colored according to atomic charges. Positively charged areas are colored in blue and negatively charged areas are colored in red.

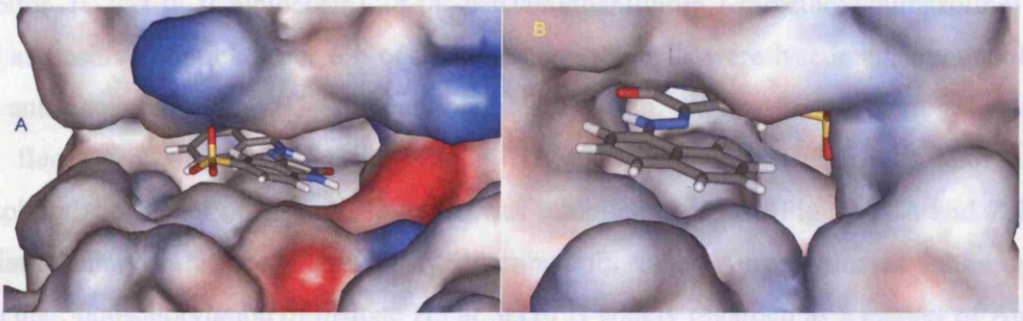
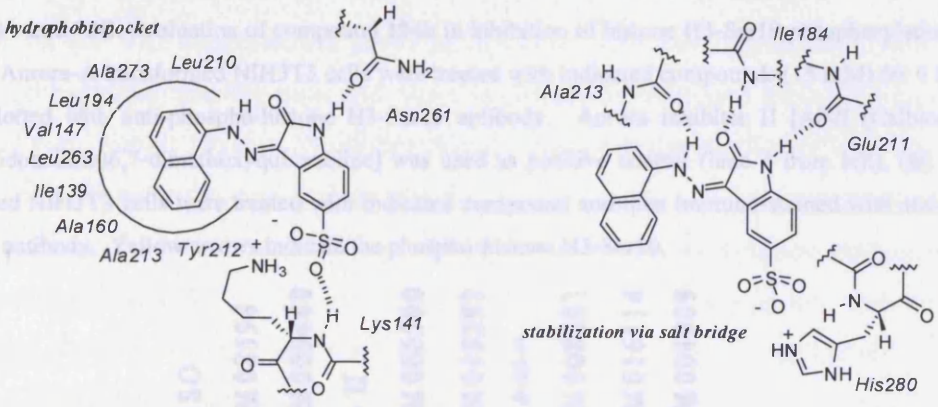


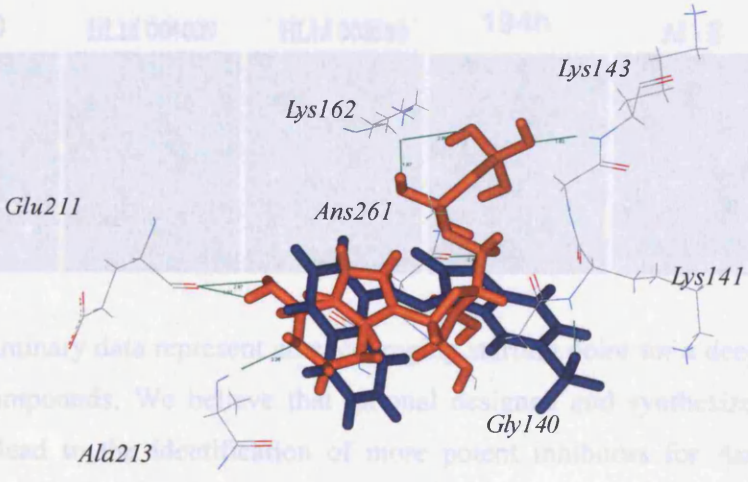
Figure 76. Schematic docking modes of **194h** with Aurora-A and Aurora-B.



194h docked to Aurora A

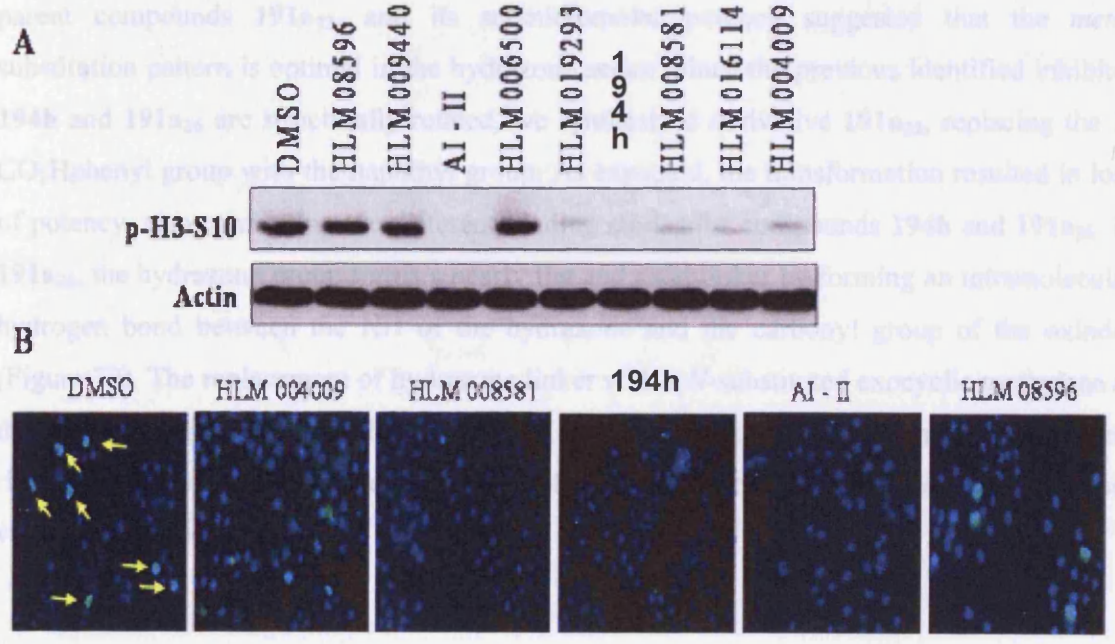
194h docked to Aurora B

Figure 77. Overlay of **194h** (coloured in blue) and ADP (coloured in brown) docked to ATP binding site.



To examine the ability of **194h** to inhibit Aurora-A kinase inside cells, Aurora-A transfected NIH3T3 cells (mouse embryonic fibroblast cell line) were treated with **194h** (15 μM). Aurora inhibitor II [AI-II (Calbiochem), 4-(4'-benzamidoanilino)6,7-dimethoxyquinazoline] was used as positive control. After 6 h of the treatment, the cells were lysed and immunoblotted with anti-phospho-histone H3-Ser10 antibody. In addition, the treated cells were also stained with fluorescence–labeled phospho-histone H3-Ser10 antibody. The results showed the phosphorylation level of histone H3-Ser10 was inhibited by **194h** (Figure 78A and 78B). A similar phenotype has been previously described for other Aurora kinases inhibitors. In particular, phosphorylation of histone H3 at Ser10 is widely regarded as a marker of Aurora-B inhibition.

Figure 78A and 78B. Evaluation of compound **194h** in inhibition of histone H3-Ser10 phosphorylation in intact cells. (**A**) Aurora-A transformed NIH3T3 cells were treated with indicated compounds (15 mM) for 6 h and then immunoblotted with anti-phospho-histone H3-Ser10 antibody. Aurora inhibitor II [AI-II (Calbiochem), 4-(4benzamidoanilino)6,7-dimethoxyquinazoline] was used as positive control (lane 4 from left). (**B**) Aurora-A transformed NIH3T3 cells were treated with indicated compound and then immuno-stained with anti-phospho-H3-Ser10 antibody. Yellow arrows indicate the phospho-histone H3-Ser10.

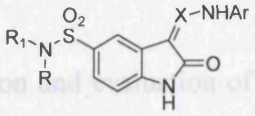


These preliminary data represent an encouraging starting point for a deeper evaluation of this class of compounds. We believe that rational designed and synthesized libraries based on **194h** can lead to the identification of more potent inhibitors for Aurora-A and –B and, therefore, enhance the selectivity. For instance, the model of **194h** docked to Aurora-A indicates that changes could be tolerated at the 6 and 7 position of the indolinone ring.

Furthermore, the model of **194h** docked to Aurora-B, shows the naphthyl group pointing out of the ATP-binding site suggesting the possibility of further substitution and modification to the naphthyl group. Finally, the mode of action of **194h** still needs to be fully addressed. Further studies are underway to confirm that in cells compound **194h** acts as an Aurora kinases inhibitor. Moreover, the antiproliferative and antitumour effect of **194h** will be assessed in a panel of different tumour cell lines.

In the second part of our project, to more fully develop the initial SAR for series **191** and **221**, we further expanded our library of sulfonamide **191** (Table 29). Compounds **191a$_{24}$** and **191a$_{23}$** were synthesized and firstly evaluated for their ability to inhibit Aurora-A activity. In an effort to improve the drug-like features of our leads, the nitro group was replaced with the carboxylic acid also capable of acting as hydrogen bonding acceptor. Not only this modification was well tolerated, but gave rise to a better Aurora-A inhibition (Table 29). The results also indicated that the bulk and more hydrophobic isopropyl group is beneficial for potency (**191a$_{24}$** versus **191a$_{23}$**). We systematically evaluated the position of the carboxyl acid group on the phenylhydrazone moiety aiming at studying the "substituent position effect". The new synthesized analogues **191a$_{26}$** displayed an enhanced potency compared to the parent compounds **191a$_{23}$**, and its submicromolar potency suggested that the *meta*-substitution pattern is optimal in the hydrazone series. Since the previous identified inhibitor **194h** and **191a$_{26}$** are structurally related, we synthesized derivative **191a$_{22}$**, replacing the 2-CO_2Hphenyl group with the naphthyl group. As expected, the transformation resulted in loss of potency, also confirming the different binding modes for compounds **194h** and **191a$_{26}$**. In **191a$_{26}$**, the hydrazone group forms a nearly flat and rigid linker by forming an intramolecular hydrogen bond between the NH of the hydrazone and the carbonyl group of the oxindol (Figure 79). The replacement of hydrazone linker with a *N*-substituted exocyclic methylene at the 3-position in compounds **191a$_{23}$**, **191a$_{26}$**, was thought to be ideal to maintain the same rigid spacer connectivity between the oxindole moiety and the benzoic acid moiety without varying the spacer length.

Table 29. *In vitro* SAR for the early key oxindole derivatives and **191** , **200**, and, **199** analogues of **HL10581**.

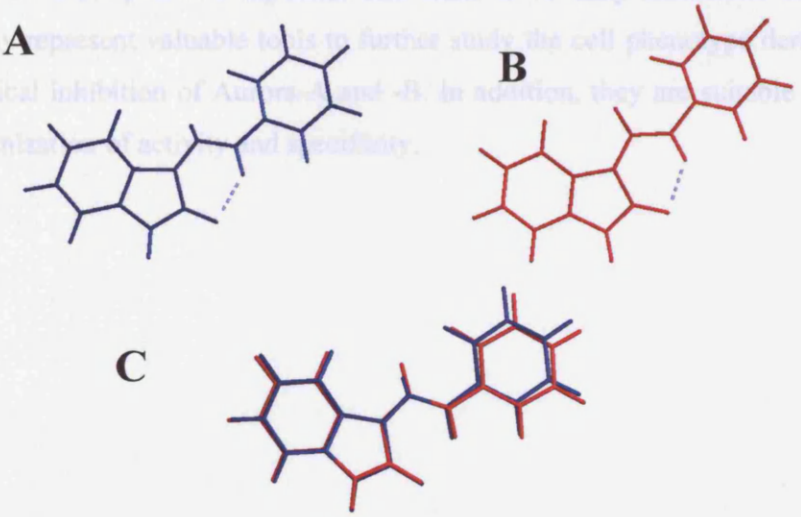


5

Entry	R	R_1	X	Ar	Aurora-A IC_{50} (μM)	Aurora-B IC_{50} (μM
191a$_{24}$	H	H	N	2-$CO_2HC_6H_4$	25	
191a$_{23}$	H	*Iso*-propyl	N	2-$CO_2HC_6H_4$	3.6	
191a$_{26}$	H	*Iso*-propyl	N	3-$CO_2HC_6H_4$	0.127	8.02
200b	H	*Iso*-propyl	CH	3-$CO_2HC_6H_4$	0.038	7.52
200c	H	*Iso*-propyl	CH	4-$CO_2HC_6H_4$	0.020	0.064
200a	H	*Iso*-propyl	CH	2-$CO_2HC_6H_4$	7	
199f[a]	H	*Iso*-propyl	CH	C_6H_5		
199g[a]	H	*Iso*-propyl	CH	1-Np		
191a$_{22}$[a]	H	*Iso*-propyl	N	1-Np		
191a$_{21}$[a]	H	*Iso*-propyl	N	C_6H_4		

a) IC_{50} values were determined only for compounds that inhibit >85% of Aurora-A kinase activity at 10 μM

Figure 79. (A) 3D representation of hydrazone. (B) 3D representation of enamine. (C) Overlay of the two representation.



An increased inhibition potency toward to Aurora-A and -B activity was observed for the new synthesized compounds **200b** and **200c** and **200a**, suggesting a preference to the more hydrophobic CH bridge over the N linker. Compounds **200b** and **200c** appear to be about 200-fold more potent than the corresponding hydrazone analogues **191a$_{23}$** and **191a$_{26}$**, respectively. As shown in Table 29, analogues with *para* and *meta* subtitution pattern are in general of similar potency, and favourable for good activity (compare **200b** and **200c** versus **200a**). Moreover, the *meta* subtitution pattern appear to be critical for Aurora-A/-B selectivity as shown by compound **191a$_{26}$** and **200b** (Table 29). The requirement of the carboxylic acid

group for optimal binding activity was further proved by the loss of potency of compounds **199f** and **191a**$_{22}$.

Further structure optimization and evaluation of the most promising compounds and further studies and analyses including cell-based assay and specificity test are underway. Finally, the mode of action of the most potent compounds still needs to be fully addressed. Moreover, these inhibitors may represent valuable tools to further study the cell phenotype deriving from the pharmacological inhibition of Aurora-A and -B. In addition, they are suitable candidates for possible optimization of activity and specificity.

10.3 Conclusion

In the search of novel Aurora kinases, we identified several hits with micromolar potency. Using these hits as starting points, we performed a series of SAR studies that determined the structural features responsible for optimal binding potency. Further lead optimization, directed to the exploration of SAR around the oxindole scaffold, led us to the identification of potent *in vitro* inhibitors of Aurora-A and -B, showing nanomolar potency. Finally, the mode of action of the most potent compounds still need to be fully addressed. Moreover these inhibitors may represent valuable tools to further study the cell phenotype deriving from the pharmacological inhibition of Aurora-A and -B. In addition, they are suitable candidates for possible optimization of activity and specificity.

Chapter 11

11.0 Experimental

11.1 General Procedures and Instrumentation

Melting points were determined using on a Barnstead international melting point apparatus and remain uncorrected. ^{1}H NMR spectra were recorded on a Bruker WM400 (400 MHz) pulsed Fourier Transform spectrometer, and a VARIAN 400 MHz, with the ^{13}C NMR spectra being recorded at 100 MHz. All coupling constants are measured in Hertz (Hz) and the chemical shifts (δ_H and δ_C) are quoted in parts per million (ppm) relative to TMS (δ 0), which is used as the internal standard. The chemial shift for ^{13}C are referenced to the solvent used. The following abbreviations are used throughout, s = singlet, d = doublet, t = triplet, dd = doublet of doublets etc. The spectra are proton decaupled. Low resolution mass spectra were determined using a Fisons VG Platform II Quadrupole instrument and Agilent Technologies LC/MSD VL instrument. High resolution mass spectroscopy was carried out by the EPSRC national mass spectrometry service centre at Swansea University, as well as on an Agilent Technologies LC/MSD (ESI-TOF) instrument at the University of South Florida, and Agilent 6210 LC/MS (ESI-TOF) at the Moffitt Cancer Centre and Research Institute. Column chromatography was performed using silica gel 60, 220-440 mesh (Apollo in the UK and Fisher in the US). Automated flash chromatography was conducted using a Flashmaster II system (Argonaut-Biotage), using Biotage silica cartridges. Thin layer chromatography waa performed using silica gel 60 F254 plates (Apollo in the UK and Fisher in the US), with observation under UV when necessary. Anhydrous solvents used as purchased: dichloromethane (anhydrous, 99.8% contains 50-150 ppm hydrocarbon as stabilizer from Aldrich), dimethyl formamide (anhydrous, 99.9% from Aldrich), tetrahydrofuran (anhydrous, 99.9%, inhibitor free, Aldrich), acetonitrile (anhydrous, 99.8%, Aldrich), toluene (anhydrous, 99.8%, Aldrich), methanol (anhydrous, 99.8%, Aldrich).

1-(6-Hydroxy-4,5,6-trimethoxyphenyl)-but-2-yn-1-one (107)[331]

Phosphorus pentoxide (1.35 g) was added to methansulfonic acid (13.5 g) and the resultant mixture stirred at room temperature under argon until the phosphorus pentoxide was dissolved. But-2-ynoic-acid (0.227 g, 2.71 mmol) was then added, followed by an equimolar amount of 3,4,5-trimethoxyphenol (**106**) (0.50 g, 2.71 mmol). The reaction mixture was degassed under argon and then stirred at room temperature for 5h. Upon complete consumption of the staring material, the dark red reaction mixture was poured slowly into saturated sodium bicarbonate solution (100 ml for every 10 g of methansulfonic acid), aqueous phase extracted with DCM (3 x 100 ml/10 g of $MeSO_3H$). The combined organic extracts were washed with brine, dried over $MgSO_4$, and the solvent removed under reduced pressure. Chromatography on silica gel (80:20 DCM/ethyl acetate 8/2, R_f 0.8) afforded pure ketone **107** (0.314 g, 1.25 mmol, 46%) as a yellow solid, mp 92-94 °C (lit[331] 92 °C). ^{1}H NMR

(400 MHz, $CDCl_3$) δ 2.11 (3H, s, CH_3), 3.72 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.16 (1H, , OH), 6.30 (1H, s, H-5).

5,6,7-Trimethoxy-2-methylchromen-4-one (108)[331]

1-(2-hydroxy-4,5,6-trimethoxyphenyl)-but-2-yn-1-one (**107**) (0.265 g, 1.06 mmol) was dissolved in dry acetone (12 ml) (the acetone was previously distilled over P_2O_5, under nitrogen), and anhydrous K_2CO_3 (0.240 g, 1.79 mmol) was then added. The resulting mixture was heated at reflux for 1h. The carbonate was removed via filtration. The filtrate was concentrated *in vacuo*. Chromatography on silica gel (70:30 DCM/ethyl acetate, R_f 0.3) afforded pure chromone **108** (0.159 g, 0.63 mmol, 60%) as a yellow solid, mp 96-99 °C (lit[331] 99 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 2.22 (3H, s, CH_3), 3.82 (3H, s, OCH_3) 3.86 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 5.94 (1H, s, H-2), 6.58 (3H, s, H-8).

Alternative route to chromenone 108[331]

Phosphorus pentoxide (13.57 g) was added to 10 g of methansulfonic acid (135.70 g) and the resultant mixture stirred at room temperature under argon until the phosphorus pentoxide was dissolved. But-2-ynoic-acid was (2.28 g, 27.12 mmol) then added. An equimolar amount of 3,4,5-trimethoxyphenol (**106**) (5.00 g, 27.12 mmol) was added immediately following the addition of the but-2-ynoic-acid, the mixture was degassed under argon and was then stirred at room temperature for 5h. Upon complete consumption of the phenol, the dark red reaction mixture was poured slowly into saturated sodium bicarbonate solution (100 ml for every 10 g of methansulfonic acid). The product was extracted with DCM (3 x 100 ml/10 g of $MeSO_3H$). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Following work up, the crude material (8.7 g) was dissolved in dry acetone (350 ml) (the acetone was distilled on P_2O_5, under nitrogen). Anhydrous K_2CO_3 (8.00 g, 58.0 mmol) was then added. The resulting mixture was heated at reflux for 1h. The carbonate was removed via filtration. The filtrate was concentrated *in vacuo*. Chromatography on silica gel (80:20 DCM/ethyl acetate, R_f 0.3) afforded pure chromone **108** (1.99 g, 7.96 mmol, 30%) as a yellow solid.

3,5-Bis(benzyloxy)benzaldehyde[410] **(222)**

3,5-Dihydroxybenzaldehyde (0.123 g, 0.89 mmol) was dissolved in DMF (3 ml) at room temperature under Ar. K_2CO_3 (0.738 g, 5.34 mmol, dried in oven at 120 °C overnight) and benzylbromide (0.335 g, 1.96 mmol) were added and the yellow reaction mixture was placed in an oil bath at 80 °C and stirred for 2 h. After cooling to room temperature, H_2O (8 ml) was added, and the aqueous phase was extracted with ethyl acetate (3 x 4 ml). The combined organic extracts were washed with brine (8 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure. Chromatography on silica gel (60:40 DCM/ethyl acetate, R_f 0.36) afforded the 3,5-bis(benzyloxy)benzaldehyde (**222**) (0.242 g,0.76 mmol, 85%) as a white

solid, mp 71-73 °C (lit[411] 80 °C). ^{1}H NMR (400 MHz, $CDCl_3$) 5.02 (4H, s, CH_2), 6.79 (1H, t, *J* 2.4 Hz, H-4), 7.04 (2H, d, *J* 2.4 Hz, H-2 & H-6), 7.27-7.37 (10H, m, ArH) 9.82 (1H, s, C<u>H</u>O).

5,6,7-Trimethoxy-2-[2-(2,5-dimethoxyphenyl)ethenyl]chromone (109c)[331]

A solution of **108** (0.135 g, 0.54 mmol) and 2,5-dimethoxybenzaldehyde (0.179 g, 1.08 mmol) was stirred in presence of sodium methoxide (0.058 g, 1.08 mmol) in methanol (5 ml) at 80 °C for 24 h. After cooling to room temperature, pure product **109c** (0.075 g, 0.19 mmol, 35%) was collected as a yellow precipitate by filtration and dried *in vacuo*, mp 153-155 °C. ^{1}H NMR ($CDCl_3$) δ 3.76 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.11 (1H, s, H-2), 6.71 (1H, d, *J* 16.4 Hz, CH), 6.74 (1H, s, H-8), 6.82-6.83 (2H, m, ArH), 7.03 (1H, d, *J* 2.8 Hz, H-6'), 7.72 (1H, d, *J* 16.4 Hz, CH). ^{13}C NMR (400 MHz, $CDCl_3$) δ 56.24 (s) (OCH_3), 56.57 (s) (OCH_3), 56.72 (s) (OCH_3), 62.42 (s) (OCH_3), 62.55 (s) (OCH_3), 96.70 (s) (<u>C</u>HCO), 111.79 (s) (CH, Ar), 112.80 (s) (CH, Ar), 113.10 (s) (C, Ar, 114.94 (s) (CH, Ar), 116.60 (s) (CH, Ar), 121.22 (s) (<u>C</u>H=CH), 125.13 (s) (CH, Ar), 131.33 (s) (CH=<u>C</u>H), 140.55 (s) (C, Ar), 152.73 (s) (C, Ar), 152.92 (s) (C, Ar), 154.06 (s) (C, Ar), 154.75 (C, Ar), 158.01 (C, Ar), 160.41 (C, Ar), 177.72 (C=O). ν_{max} (solid)/(cm^{-1}) 1665 (st), 1578 (st), 1446 (st), 1421 (st), 1223 (st), 1119. MS *m/z* (**API-ES**): found 399 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 399.1472 $(M+H)^+$, calculated for $C_{22}H_{23}O_7$ 399.1444; found 421.1268 $(M+Na)^+$, calculated for $C_{22}H_{22}NaO_7$ 421.1263.

***5,6,7-Trimethoxy-2-[2-(3,5-dimethoxyphenyl)ethenyl]chromone* (109a).[331]** This was prepared from **108** (0.144 g, 0.58 mmol) and 3,5-dimethoxybenzaldehyde (0.192 g. 1.15 mmol) in a similar manner as described for preparation of **109c**, reaction time 48 h. Chromatography on silica gel (60:40 ethyl acetate/petroleum ether, R_f 0.30) afforded pure **109a** as a white solid (0.057 g, 0.14 mmol, 25%) mp 148-150 °C (lit[331] 148.5-151 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (6H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.16 (1H, s, H-2), 6.42 (2H, t, *J* 2.4 Hz, H-4'), 6.63 (2H, d, *J* 2.4 Hz, H-2' & H-6'), 6.63 (1H, d, *J* 16.0 Hz, CH), 6.72 (1H, s, H-8), 7.37 (1H, d, *J* 16.0 Hz, CH).

***5,6,7-Trimethoxy-2-[2-[3,5-bis(benzyloxy)phenyl]ethenyl]chromone* (109b).[331]** This was prepared from **98** (0.125 g, 0.50 mmol) and 3,5-bis(benzyloxy)benzaldehyde **222** (0.318 g, 1.0 mmol) in a similar manner as described for preparation of **109c**, reaction time 24 h. Chromatography on silica gel (60:40 ethyl acetate/petroleum ether, R_f 0.26) afforded pure **109b** as a white solid (0.116 g, 0.21 mmol, 42%), mp 160-161 °C (lit[331] 165-165.5 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.84 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 5.02 (3H, s, CH_2), 6.14 (1H, s, H-2), 6.58 (1H, s, H, H-8), 6.62 (1H, d, 15.2 Hz, CH), 6.71-6.73 (3H, m, ArH), 7.26-7.38 (11H, m, ArH & CH).

5,6,7-Trimethoxy-2-[2-(2,4,5-dimethoxyphenyl)ethenyl]chromone **(109d)**. This was obtained as a yellow solid (0.053 g, 0.12 mmol, 30%) from **108** (0.108 g, 0.43 mmol) and 2,4,5-trimethoxybenzaldehyde (0.169 g, 0.86 mmol) in a similar manner as described for preparation of **109c**, reaction time 24 h, mp 161-163 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 6.14 (1H, s, H-2), 6.56 (1H, s, H-8), 6.67 (1H, d, *J* 16.0 H, CH), 6.81 (1H, s, ArH), 7.08 (1H, s, ArH), 7.79 (1H, d, *J* 16.0 H, CH). ^{13}C NMR (100 MHz, $CDCl_3$ δ 56.47 (OCH_3), 56.61 (OCH_3), 56.84 (OCH_3), 56.93 (OCH_3), 61.93 (OCH_3), 62.54 (OCH_3), 96.66 (CHCO), 97.31 (CH, Ar), 110.62 (CH, Ar), 110.87 (CH, Ar), 113.41 (C, Ar), 116.07 (C, Ar), 118.19 (CH=CH), 131.11 (CH=CH), 140.47 (C, Ar), 140.79 (C, Ar), 151.96 (C, Ar), 152.89 (C, Ar), 153.68 (C, Ar), 154.74 (C, Ar), 157.91 (C, Ar), 161.02 (C, Ar), 177.78 (C=O). ν_{max} (nujol)/(cm^{-1}) 1642 (st), 1599 (st), 1455 (st), 1416 (st), 1202 (st), 1113 (st), 1026 (st). MS *m/z* (**API-ES**): found 429 $(M+H)^+$ (100 %). HRMS *m/z* (**API-ES**): found 429.1574 $(M+H)^+$, calculated for $C_{23}H_{25}O_8$ 429.1549

5,6,7-Trimethoxy-2-[2-(4-chlorophenyl)ethenyl]chromone **(109e)**. This was obtained as a white solid (0.070 g, 0.188 mmol, 44%) from **108** (0.108 g, 0.43 mmol) and 4-chlorobenzaldehyde (0.121 g, 0.86 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 190-192 °C; ^{1}H NMR ($CDCl_3$) δ 3.84 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.12 (1H, s, H-2), 6.63 (1H, d, *J* 16.0 Hz, CH), 6.71 (1H, s, H-8), 7.32 (2H, d, 8.0 Hz, 2 x CH), 7.40 (1H, d, *J* 16.0 Hz, CH), 7.43 (2H, d, 8.0 Hz, 2 x C). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.70 (OCH_3), 61.95 (OCH_3), 62.56 (OCH_3), 96.50 (CHCO), 112.24 (CH, Ar), 113.47 (C, Ar), 120.96 (CH=CH), 129.05 (2 x CH, Ar), 129.62 (2 x CH, Ar), 134.01 (C, Ar), 134.81 (CH=CH), 135.84 (C, Ar), 140.65 (C, Ar), 152.98 (C, Ar), 154.66 (C, Ar), 158.17 (C, Ar), 159.50 (C, Ar), 177.57 (C=O); ν_{max} (nujol)/(cm^{-1}) 2853 (st), 1645 (sr), 1436 (st), 1375 (st). MS *m/z* (**API-ES**): found 373, $(M^{35}Cl+H)^+$ (100%), 375 $(M^{37}Cl+H)^+$ (35%). HRMS *m/z* (**API-ES**): found 373.0841 $(M+H)^+$, calculated for $C_{20}H_{18}ClO_5$ 373.0843; found 395.0663 $(M+Na)^+$, calculated for $C_{20}H_{17}ClNaO_5$ 395.0662.

5,6,7-Trimethoxy-2-[2-(3-chlorophenyl)ethenyl]chromone **(109f)**. This was obtained as a white solid (0.093 g, 0.24 mmol, 48%) from **108** (0.130 g, 0.52 mmol) and 4-chlorobenzaldehyde (0.146 g, 1.04 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 195-197 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94(3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 6.32 (1H, s, H-2), 6.76 (1H, d, *J* 16.0 Hz, CH), 6.81 (1H, s, H-8), 7.37 (2H, m, ArH), 7.46 (1H, m, ArH), 7.49 (1H, d, *J* 16.0 Hz, CH), 7.57 (1H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.75 (OCH_3), 61.85 (OCH_3) 62.91 (OCH_3), 96.89 (CHCO), 112.13 (CH, Ar), 113.61 (C, Ar), 121.42, (CH=CH) 125.59 (CH, Ar), 127.39 (CH, Ar), 129.30 (CH, Ar), 130.21 (CH, Ar), 131.04 (C, Ar), 132.07 (C, Ar), 133.88 (C, Ar), 134.24 (CH=CH), 140.57 (C, Ar), 151.10 (C, Ar), 154.98 (C, Ar), 158.36 (C, Ar),

159.40 (C, Ar), 177.58 (C=O). ν_{max} (nujol)/(cm^{-1}) 2853 (st), 1649 (st), 1465 (st), 1378 (st). MS *m/z* (**API-ES**): found 373, $(M^{35}Cl+H)^+$ (100%), 375 $(M^{37}Cl+H)^+$ (30%). HRMS *m/z* (**API-ES**): found 373.0844 $(M+H)^+$, calculated for $C_{20}H_{18}ClO_5$ 373.0843; found 395.0664 $(M+Na)^+$, calculated for $C_{20}H_{17}ClNaO_5$ 395.0662.

***5,6,7-Trimethoxy-2-[2-(2-chlorophenyl)ethenyl]chromone* (109g)**. This was obtained as a white solid (0.156 g, 0.41 mmol, 43%) from **108** (0.102 g, 0.40 mmol) and 4-chlorobenzaldehyde (0.114 g, 0.81 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 155-157 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.15 (1H, s, H-2), 6.71 (1H, d, *J* 16.0 Hz, CH), 6.73 (1H, s, H-8), 7.23-7.25 (2H, m, ArH), 7.36-7.43 (1H, m, ArH), 7.61-7.63 (1H, m, ArH), 7.85 (1H, d, *J* 16.0 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.79 (OCH_3), 61.95 (OCH_3), 62.56 (OCH_3), 96.79 (CHCO), 112.64 (CH, Ar), 113.48 (C, Ar), 122.94 (CH=CH), 127.45 (CH, Ar), 127.61 (CH, Ar), 130.57 (CH, Ar), 130.84 (CH, Ar), 132.05 (CH=CH), 133.73 (C, Ar), 134.83 (C, Ar), 140.67 (C, Ar), 150.91 (C, Ar), 154.70 (C, Ar), 158.23 (C, Ar), 159.44 (C, Ar), 177.60 (C=O). ν_{max} (nujol)/(cm^{-1}) 2853 (st), 1654 (st), 1460 (st), 1378 (st). MS *m/z* (**API-ES**): found 373 $(M^{35}Cl+H)^+$ (100%), 375 $(M^{37}Cl+H)^+$ (30%). HRMS *m/z* (**API-ES**): found 373.0844 $(M+H)^+$, calculated for $C_{20}H_{18}ClO_5$ 373.0843; found 395.0663 $(M+Na)^+$, calculated for $C_{20}H_{17}ClNaO_5$ 395.0662.

***5,6,7-Trimethoxy-2-[2-(3,4-dichlorophenyl)ethenyl]chromone* (109h)**. This was obtained as a yellow solid (0.094 g, 0.23 mmol, 45%) from **108** (0.127 g, 0.51 mmol) and 3,4-dichlorobenzaldehyde (0.178 g, 1.02 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 221-223 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 6.24 (1H, s, H-2), 6.73 (1H, d, *J* 16.2 Hz, CH), 6.79 (1H, s, H-8), 7.12 (1H, dd, *J* 2.5, 7.7 Hz, H-6'), 7.42 (1H, d, *J* 16.2 Hz, CH), 7.50 (1H, 1H, d, *J* 7.7 Hz, H-5') 7.67 (1H, 1H, d, *J* 2.5 Hz, H-2'). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.07 (OCH_3), 61.95 (OCH_3), 62.58 (OCH_3), 96.48 (CHCO), 112.70 (CH, Ar), 113.05 (C, Ar), 122.19 (CH=CH), 126.75 (CH, Ar), 129.56 (CH, Ar), 131.32 (CH, Ar), 133.47 (CH=CH), 133.66 (C, Ar), 133.85 (C, Ar), 133.55 (C, Ar), 141.20 (C, Ar), 150.61 (C, Ar), 154.62 (C, Ar), 158.25 (C, Ar), 159.00 (C, Ar), 177.47 (C=O). ν_{max} (nujol)/(cm^{-1}) 2853 (st), 1649 (st), 1465 (st), 1377 (st). MS *m/z* (**API-ES**): found 407 $(M^{35}Cl+H)^+$ (100%), 409 $(M^{37}Cl+H)^+$ (70%). HRMS *m/z* (**API-ES**): found 407.0415 $(M+H)^+$, calculated for $C_{20}H_{17}Cl_2O_5$ 407.0453; found 429.0328 $(M+Na)^+$, calculated for $C_{20}H_{16}Cl_2NaO_5$ 429.0272.

***5,6,7-Trimethoxy-2-[2-(2,4-dichlorophenyl)ethenyl]chromone* (109i)**. This was obtained as a yellow solid (0.089 g, 0.22 mmol, 41%) from **108** (0.135 g, 0.54 mmol) and 2,4-dichlorobenzaldehyde (0.189 g, 1.08 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 214-216 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (3H, s,

OCH_3), 3.90 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.18 (1H, s, H-2), 6.63 (1H, d, *J* 16.4 Hz, CH), 7.24 (1H, dd, *J* 2.0, 8.6 Hz, H-5'), 7.40 (1H, d, *J* 2.0 Hz, H-3'), 7.55 (1H, d, *J* 8.6 Hz, H-6'), 7.77 (1H, d, *J* 16.4 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.79 (OCH_3), 61.94 (OCH_3), 62.56 (OCH_3), 96.65 (CHCO), 112.88 (CH, Ar), 113.48 (C, Ar), 123.35 (CH=CH), 128.07 (CH, Ar), 128.17 (CH, Ar), 130.37 (CH, Ar), 130.80 (CH, Ar), 132.34 (CH=CH), 135.31 (C, Ar), 136.02 (C, Ar), 140.72 (C, Ar), 152.95 (C, Ar), 154.66 (C, Ar), 158.29 (C, Ar), 159.10 (C, Ar), 177.52 (C=O). ν_{max} (nujol)/(cm^{-1}) 2853 (st), 1650 (st), 1488 (st), 1356 (st). MS *m/z* (**API-ES**): found 407 (M ^{35}Cl+H)$^+$ (100%), 409 (M ^{37}Cl+H)$^+$ (70%). HRMS *m/z* (**API-ES**): found 407.0424 (M+H)$^+$, calculated for $C_{20}H_{17}Cl_2O_5$ 407.0453; found 429.0258 (M+Na)$^+$, calculated for $C_{20}H_{16}Cl_2NaO_5$ 429.0272.

5,6,7-Trimethoxy-2-[2-(2,6-dichlorophenyl)ethenyl]chromone **(109j)**. This was obtained as a white solid (0.066 g, 0.162 mmol, 41%) from **108** (0.100 g, 0.400 mmol) and 2,6-dichlorobenzaldehyde (0.140 g, 0.080 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 175-177 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.15 (1H, s, H-2), 6.72 (1H, s, H-8), 6.81 (1H, d, *J* 16.6 Hz, CH), 7.13 (1H, t, *J* 8.4, Hz, H-4'), 7.32 (2H, d *J* 2.0 Hz, H-3' & H5'), 7.51 (1H, d, *J* 16.6 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.77 (OCH_3), 61.96, (OCH_3), 62.56 (OCH_3), 96.69 (CHCO), 113.110 (C, Ar), 113.54 (CH, Ar), 128.97 (CH=CH), 129.26 (2 x CH, Ar), 129.28 (C, Ar), 129.87 (CH, Ar), 132.96 (C), 135.17 (CH=CH), 140.70 (C, Ar), 152.95 (C, Ar), 154.72 (C, Ar), 158.26 (C, Ar), 158.84 (C, Ar), 177.59 (C=O). ν_{max} (nujol)/(cm^{-1}) 2853 (st), 1651 (st), 1463 (st), 1377 (st). MS *m/z* (**API-ES**): found 407 (M^{35}Cl+H)$^+$ (100%), 409 (M^{37}Cl+H)$^+$ (70%). HRMS *m/z* (**API-ES**): found 407.0458 (M+H)$^+$, calculated for $C_{20}H_{17}Cl_2O_5$ 407.0453; found 429.0281 (M+Na)$^+$, calculated for $C_{20}H_{16}Cl_2NaO_5$ 429.0272.

5,6,7-Trimethoxy-2-[2-(4-nitrophenyl)ethenyl]chromone **(109k)**. This was obtained as a yellow solid (0.061 g, 0.160 mmol, 40%) from **108** (0.100 g, 0.40 mmol) and 4-nitrobenzaldehyde (0,120 g, 0.79 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 187-189 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.18 (1H, s, H-2), 6.72 (1H, s, H-8), 6.80 (1H, d, *J* 16.0 Hz, CH), 7.49 (1H, d, *J* 16.0 Hz, CH), 7.65 (2H, d, *J* 8.7 Hz, 2 x CH, Ar), 8.20 (2H, d, *J* 8.7 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.74 (OCH_3), 61.95 (OCH_3), 62.57 (OCH_3), 96.50 (CHCO), 113.52 (CH, Ar), 124.70 (2 x CH, Ar), 128.42 (2 x CH, Ar), 133.33 (CH=CH), 140.82 (C, Ar), 141.70 (C, Ar), 148.33 (C, Ar), 153.02 (C, Ar), 154.62 (C, Ar), 158.41 (C, Ar), 158.55 (C, Ar), 177.04 (C=O). ν_{max} (nujol)/(cm^{-1}) 2855 (st), 1630 (st), 1480 (st), 1340 (st). MS *m/z* (**API-ES**): found 384 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 384.1087 (M+H)$^+$, calculated for $C_{20}H_{18}NO_7$ 384.1083; found 406.0908 (M+Na)$^+$, calculated $C_{20}H_{17}NNaO_7$ 406.0903.

2,3,4-Trimethoxyphenol (111)[332]

2,3,4-Trimethoxybenzaldehyde (**110**) (7.6 g, 38.73 mmol) and H_2O_2 (aq, 33% solution) (6.13 g 19.57 mmol) were stirred in the presence of concd. H_2SO_4 (0.77 ml) in methanol (80 ml) under nitrogen at room temperature for 1 h. Triethylamine (2 ml) was added, and the solvent removed under reduced pressure. Water (80 ml) was added and aqueous phase extracted with DCM (3 x 80 ml). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Chromatography on silica gel (50:50 hexane/ethyl acetate, R_f 0.68) afforded pure **111** (6.74 g, 36.59 mmol, 94%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.32 (1H, bs, OH), 6.48 (1H, d, *J* 9.2 Hz, ArH), 6.56 (1H, d, *J* 9.2 H, ArH).

6,7,8-trimethoxy-2-methylchromen-4-one (112)[331]

Phosphorus pentoxide (2.71 g) was added to methansulfonic acid (27.15 g) and the resultant mixture stirred at room temperature under argon until the phosphorus pentoxide was dissolved. But-2-ynoic-acid (0.457 g, 5.43 mmol) was then added, followed by an equimolar amount of **111** (1.01 g, 5.43 mmol). The reaction mixture was degassed under argon and then stirred at room temperature for 5h. Upon complete consumption of the staring material, the dark red reaction mixture was poured slowly into saturated sodium bicarbonate solution (100 ml for every 10 g of methansulfonic acid), and the aqueous phase extracted with DCM (3 x 100 ml/10 g of $MeSO_3H$). The combined organic extracts were washed with brine, dried over $MgSO_4$, and the solvent removed under reduced pressure. Chromatography on silica gel (80:20 DCM/ethyl acetate, R_f 0.30) afforded pure chromone **112** (0.166 g, 0.664 mmol, 12%) as an orange solid, mp 94-99 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (3H, s, CH_3), 3.87 (3H, s OCH_3), 3.94 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.01 (1H, s, CH), 7.06 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.29 (CH_3), 56.49 (OCH_3), 60.89 (OCH_3), 61.49 (OCH_3), 100.52 (CHCO), 111.45 (CH, Ar), 112.78 (C, Ar), 141.09 (C, Ar), 153.02 (C, Ar), 155.09 (C, Ar), 158.12 (C, Ar), 162.96 (C, Ar), 176.64 (C=O). ν_{max} (nujol)/(cm^{-1}) 2865 (st), 1654 (st), 1589 (st). MS *m/z* (**API-ES**): found 251 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 251.0918 $(M+H)^+$, calculated for $C_{13}H_{15}O_5$ 251.0919

6,7,8-Trimethoxy-2-[2-(2-chlorophenyl)ethenyl]chromone **(113a).** This was obtained as a yellow solid (0.083 g, 0.22 mmol, 59%) from **112** (0.094 g, 0.37 mmol) and 2-chlorobenzaldehyde (0,106 g, 1.24 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 151-153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 6.28 (1H, s, H-2), 6.74 (1H, d, *J* 16.0 Hz, CH), 7.23-7.29 (3H, m), 7.29 (1H, s, H-5), 7.31-7.40 (1H, m, ArH), 7.64-7.67 (1H, m, ArH), 8.04 (1H, d, *J* 16.0 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.67 (OCH_3), 61.93 (OCH_3), 62.49 (OCH_3), 100.38 (CHCO), 110.82 (CH, Ar), 120.26 (C, Ar), 122.94 (CH=CH), 127.37 (CH, Ar), 127.60 (CH, Ar), 130.61 (CH, Ar), 131.00 (CH, Ar), 132.85 (CH=CH), 133.49

(C, Ar), 135.08 (C, Ar), 142.23 (C, Ar), 146.06 (C, Ar), 147.91 (C, Ar), 151.40 (C, Ar), 161.18 (C, Ar), 178.12 (C=O). ν_{max} (solid)/(cm^{-1}) 2861(st), 1649 (st), 1463 (st), 1369 (st). MS *m/z* (**API-ES**): found 372 $(M^{35}Cl+H)^+$ (100%), 373 $(M^{37}Cl+H)^+$ (35%). HRMS *m/z* (**API-ES**): found 373.0847 $(M+H)^+$, calculated for $C_{20}H_{18}ClO_5$ 373.0843; found 395.0667 $(M+Na)^+$, calculated for $C_{20}H_{17}ClNaO_5$ 395.0662.

6,7,8-Trimethoxy-2-[2-(3-chlorophenyl)ethenyl]chromone **(113b)**. This was obtained as a yellow solid (0.129 g, 0.34 mmol, 56%) from **101** (0.150 g, 0.61 mmol) and 3-chlorobenzaldehyde (0.174 g, 1.23 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 161-162 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 3.89 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 6.25 (1H, s, H-2), 6.74 (1H, d, *J* 16.2 Hz, CH), 7.28-7.30 (3H, m, ArH), 7.38-7.41 (1H, m, ArH), 7.47 (1H, d, *J* 16.2 Hz, CH), 7.50 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.68 (OCH_3), 61.89 (OCH_3), 62.49 (OCH_3), 100.45 (CHCO), 110.83 (CH, Ar), 120.35 (C, Ar), 122.24 (CH=CH), 126.19 (CH, Ar), 127.83 (CH, Ar), 130.02 (CH, Ar), 130.60 (CH, Ar), 135.32 (CH=CH), 135.40 (C, Ar), 137.24 (C, Ar), 142.27 (C, Ar), 145.92 (C, Ar), 147.93 (C, Ar), 151.48 (C, Ar), 161.07 (C, Ar), 178.04 (C=O). ν_{max} (solid)/(cm^{-1}) 2848 (st), 1661 (st), 1443 (st), 1387 (st). MS *m/z* (**API-ES**): found 372 $(M^{35}Cl+H)^+$ (100%), 373 $(M^{37}Cl+H)^+$ (35%). HRMS *m/z* (**API-ES**): found 373.0847 $(M+H)^+$, calculated for $C_{20}H_{18}ClO_5$ 373.0843; found 395.0667 $(M+Na)^+$, calculated for $C_{20}H_{17}ClNaO_5$ 395.0662.

6,7,8-Trimethoxy-2-[2-(4-chlorophenyl)ethenyl]chromone **(113c)**. This was obtained as a pink solid (0.060 g, 0.16 mmol, 58%) from **112** (0.068 g, 0.270 mmol) and 4-chlorobenzaldehyde (0.077 g, 0.55 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 166-168 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 3.73 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 6.08 (1H, s, H-2), 6.55 (1H, d, *J* 16. 0 Hz, CH), 7.14 (1H, s, H-5), 7.17 (2H, d, *J* 8.2 Hz, 2 x CH, Ar), 7.30 (2H, d, *J* 8.2 Hz, 2 x CH, Ar), 7.33 (1H, d, *J* 16. 0 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.66 (OCH_3), 61.88 (OCH_3), 62.49 (OCH_3), 100.46 (CHCO), 110.53 (CH, Ar), 120.32 (C, Ar), 121.35 (CH=CH), 129.21 (2 x CH, Ar), 129.62 (2 x CH, Ar), 133.90 (C, Ar), 135.54 (C, Ar), 136.01 (CH=CH), 142.25 (C, Ar), 145.90 (C, Ar), 147.89 (C, Ar), 151.45 (C, Ar), 161.34 (C, Ar), 178.07 (C=O). ν_{max} (nujol)/(cm^{-1}) 2853 (st), 1662 (st), 1455 (st), 1378 (st). MS *m/z* (**API-ES**): found 372 $(M^{35}Cl+H)^+$ (100%), 373 $(M^{37}Cl+H)^+$ (35%). HRMS *m/z* (**API-ES**): found 373.0845 $(M+H)^+$, calculated for $C_{20}H_{18}ClO_5$ 373.0843; found 395.0665 $(M+Na)^+$, calculated for $C_{20}H_{17}ClNaO_5$ 395.0662.

6,7,8-Trimethoxy-2-[2-(2,4-dichlorophenyl)ethenyl]chromone **(113d)**. This was obtained as a white solid (0.032 g, 0.08 mmol, 63%) from **112** (0.032 g, 0.128 mmol) and 2,3-dichlorobenzaldehyde (0.044 g, 0.25 mmol) in a similar manner as described for preparation

of **109c**, reaction time 12 h, mp 175-177 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.82 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.20 (1H, s, H-2), 6.65 (1H, d, *J* 16.0 Hz, CH), 7.18 (1H, dd, *J* 2.2, 8.6 Hz, H-5'), 7.23 (1H, s, H-5), 7.35 (1H, d, *J* 2.2 Hz, H-3'), 7.52 (1H, d, *J* 8.6 Hz, H-6'), 7.88 (1H, d, *J* 16.0 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.67 (OCH_3), 61.92 (OCH_3), 62.47 (OCH_3), 100.39 (CHCO), 111.06 (CH, Ar), 120.25 (CH=CH), 123.36 (C, Ar), 128.07 (2 x CH, Ar), 130.42 (CH, Ar), 131.56 (CH=CH), 132.09, (C, Ar) 135.55 (C, Ar), 136.20 (C, Ar), 142.21 (C, Ar), 146.01 (C, Ar), 148.02 (C, Ar), 151.46 (C, Ar), 160.83 (C, Ar), 178.05 (C=O). ν_{max} (nujol)/(cm^{-1}) 2854 (st), 1656 (st), 1461 (st), 1375 (st). MS *m/z* (**API-ES**): found 407 $(M^{35}Cl+H)^+$ (100%), 408 $(M^{37}Cl+H)^+$ (70%). HRMS *m/z* (**API-ES**): found 407.0463 $(M+H)^+$, calculated for $C_{20}H_{17}Cl_2O_5$ 407.0453; found 429.0285 $(M+Na)^+$, calculated for $C_{20}H_{16}Cl_2NaO_5$ 429.0272.

6,7,8-Trimethoxy-2-[2-(2,6-dichlorophenyl)ethenyl]chromone **(113e)**. This was obtained as a white solid (0.067 g, 0.16 mmol, 65%) from **112** (0.063 g, 0.25 mmol) and 2,3-dichlorobenzaldehyde (0.088 g, 0.50 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 182-184 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 6.26 (1H, s, H-2), 6.95 (1H, d, *J* 16.4 Hz, CH), 7.14 (1H, t, *J* 8.2 Hz, H-4'), 7.30 (1H, s, H-5), 7.33 (2H, d, *J* 8.2 Hz, H-3' & H-5'), 7.71 (1H, d, *J* 16.4 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.66 (OCH_3), 61.92 (OCH_3), 62.48 (OCH_3), 100.41 (CHCO), 111.49 (CH, Ar), 120.31 (C, Ar), 128.95 (CH=CH), 129.33 (2 x CH, Ar), 129.98 (CH, Ar), 130.59 (CH=CH), 132.62 (C, Ar), 135.32 (C, Ar), 142.28 (C, Ar), 146.09 (C, Ar), 148.02 (C, Ar), 151.44 (C, Ar), 160.55 (C, Ar), 178.13 (C=O). ν_{max} (nujol)/(cm^{-1}) 2857 (st), 1653 (st), 1460 (st), 1380 (st). MS *m/z* (**API-ES**): found 407 $(M^{35}Cl+H)^+$ (100%), 408 $(M^{37}Cl+H)^+$ (70%). HRMS *m/z* (**API-ES**): found 407.0465 $(M+H)^+$, calculated for $C_{20}H_{17}Cl_2O_5$ 407.0453; found 429.0291 $(M+Na)^+$, calculated for $C_{20}H_{16}Cl_2NaO_5$ 429.0272.

6,7,8-Trimethoxy-2-[2-(3,4-dichlorophenyl)ethenyl]chromone **(113f)**. This was obtained as a white solid (0.056 g, 0.138 mmol, 87%) from **112** (0.040 g, 0.159 mmol) and 2,3-dichlorobenzaldehyde (0.055 g, 0.318 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 188-190 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 6.25 (1H, s, H-2), 6.72 (1H, d, *J* 16.0 Hz, CH), 7.29 (1H, s, H-5), 7.34-7.36 (1H, m, ArH), 7.40-7.44 (2H, m, ArH & CH), 7.60 (1H, d, 1.2 Hz, H-2'). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.68 (OCH_3), 61.89 (OCH_3), 62.50 (OCH_3), 100.45 (CHCO), 111.03 (CH, Ar), 120.34 (C, Ar), 122.64 (CH=CH), 126.96 (CH, Ar), 129.62 (CH, Ar), 131.33 (CH, Ar), 133.71 (C, Ar), 133.98 (C, Ar), 134.16 (C, Ar), 135.47 (CH=CH), 142.27 (C, Ar), 145.89 (C, Ar), 147.98 (C, Ar), 151.54 (C, Ar), 160.80 (C, Ar), 178.01 (C=O). ν_{max} (nujol)/(cm^{-1}) 2853 (st), 1656 (st), 1463 (st), 1375 (st). MS *m/z* (**API-ES**): found 407 $(M^{35}Cl+H)^+$ (100%), 408 $(M^{37}Cl+H)^+$ (70%). HRMS *m/z* (**API-ES**): found

407.0453 $(M+H)^+$, calculated for $C_{20}H_{17}Cl_2O_5$ 407.0453; found 429.0275 $(M+Na)^+$, calculated for $C_{20}H_{16}Cl_2NaO_5$ 429.0272.

6,7,8-Trimethoxy-2-[2-(3,5-dimethoxyphenyl)ethenyl]chromone **(113g).** This was obtained as a yellow solid (0.093 g, 0.24 mmol, 37%) from **112** (0.158 g, 0.63 mmol) and 3,5-dimethoxybenzaldehyde (0.210 g, 1.26 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 183-185 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (6H, s, 2 x OCH_3), 3.96 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 6.31 (1H, s, H-2), 6.50 (1H, t, *J* 2.2 Hz, H-4'), 6.72 (1H, d, *J* 2.2 Hz, H-2' H-6'), 6.77 (1H, d, *J* 16 Hz, CH). 7.37 (1H, s, H-5), 7.53 (1H, d, *J* 16 Hz, CH) ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.52 (2 x OCH_3), 56.29 (OCH_3), 61.51 (OCH_3), 62.15 (OCH_3), 100.07 (CHCO), 102.00 (CH, Ar), 105.64 (2 x CH, Ar), 110.04 (CH, Ar), 119.97 (C, Ar), 120.92 (CH=CH), 136.65 (CH=CH), 136.91 (C, Ar), 141.87 (C, Ar), 145.55 (C, Ar), 147.46 (C, Ar), 151.01 (C, Ar), 161.11 (C, Ar), 161.15 (C, Ar), 177.70 (C=O). ν_{max} (solid)/(cm^{-1}) 2930 (st), 1642 (st), 1603 (st), 1585 (st), 1466 (st), 1424 (st), 1385 (st), 1194 (st), 1119 (st). MS *m/z* (**API-ES**): found 399 $(M+H)^+$ (100 %). HRMS *m/z* (**API-ES**): found 399.1442 $(M+H)^+$, calculated for $C_{22}H_{23}O_7$ 399.1444.

6,7,8-Trimethoxy-2-[2-(2,4,5-trimethoxyphenyl)ethenyl]chromone **(113h).** This was obtained as a yellow solid (0.082 g, 0.19 mmol, 37%) from **112** (0.136 g, 0.54 mmol) and 2,4,5-trimethoxybenzaldehyde (0.213 g, 1.08 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 191-193 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.94(3H, s, OCH_3), 3.95 (6H, s, 2 x OCH_3), 3.96 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.14 (3H, s, OCH_3), 6.31 (1H, s, H-2), 6.55 (1H, s, ArH), 6.77 (1H, d, *J* 16.0 Hz, CH'), 7.09 (1H, s, ArH). 7.38 (1H, s, H-5), 7.99 (1H, d, *J* 16.0 Hz, CH) ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.07 (OCH_3), 56.25 (OCH_3), 56.51 (OCH_3), 61.52 (2 x OCH_3), 62.08 (OCH_3), 93.86 (CH, Ar), 100.03 (CHCO), 108.65 (CH, Ar), 110.02 (CH, Ar), 1115.56 (C, Ar), 117.73 (CH=CH), 119.96 (C, Ar), 131.56 (CH=CH), 141.79 (C, Ar), 143.39 (C, Ar), 145.54 (C, Ar), 147.17 (C, Ar), 150.76 (C, Ar), 151.76 (C, Ar), 153.47 (C, Ar), 162.52 (C, Ar), 177.70 (C=O). ν_{max} (solid)/(cm^{-1}) 2938 (st), 1637 (st), 1586 (st), 1468 (st), 1425 (st), 1375 (st), 1210 (st), 1112 (st), 1026 (st). MS *m/z* (**API-ES**): found 429 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 429.1574 $(M+H)^+$, calculated for $C_{23}H_{25}O_8$ 429.1549

6,7,8-Trimethoxy-2-[2-(2,5-dimethoxyphenyl)ethenyl]chromone **(113i).** This was obtained as a yellow solid (0.073 g, 0.18 mmol, 34%) from **112** (0.135 g, 0.54 mmol) and 2,5-dimethoxybenzaldehyde (0.179 g, 1.08 mmol) in a similar manner as described for preparation of **109c**, reaction time 12 h, mp 145-148 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.85 (3H, s, OCH_3), 3.91 (6H, s, 2 x OCH_3), 3.97 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 4.13 (3H, s, OCH_3), 6.34 (1H, s, H-2), 6.97-7.02 (3H, s, 2 x ArH & CH), 7.13 (1H, d, *J* 2.4 Hz, H-6'),

7.39 (1H, s, H-5), 7.96 (1H, d, *J* 16.0 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.88 (OCH_3), 56.23 (OCH_3), 56.29 (OCH_3), 61.55 (OCH_3), 62.10 (OCH_3), 100.03 (CHCO), 109.55 (CH, Ar), 112.44 (CH, Ar), 112.79 (CH, Ar), 116.42 (CH, Ar), 119.96 (C, Ar), 120.90 (CH=CH), 124.60 (C, Ar), 131.95 (CH=CH), 141.85 (C, Ar), 145.63 (C, Ar), 147.34 (C, Ar), 150.89 (C, Ar), 152.51 (C, Ar), 153.63 (C, Ar), 161.94 (C, Ar), 177.80 (C=O). ν_{max} (solid)/(cm^{-1}) 2943 (st), 1648 (st), 1631 (md), 1499 (st), 1463 (st), 1424 (st) 1227 (st), 1026 (st). MS *m/z* (**API-ES**): found 399 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 399.1472 $(M+H)^+$, calculated for $C_{22}H_{23}O_7$ 399.1444.

1,2,3,4-Tetramethoxybenzene (114)[334]

2,3,4-Trimethoxy phenol (**111**) (6.4 g, 35 mmol) dimethyl sulphate (4.8 g, 38 mmol), K_2CO_3 (13 g, 94 mmol) in dry acetone (45 ml) was refluxed for 22h. The solvent was removed under reduced pressure and H_2O (45 ml) was added. Pure **114** was collected by filtration and dried *in vacuo* (6.8 g, 34.32 mmol, yield 99%), mp (MeOH) 88-89 °C (lit[334] 91 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 3.85 (6H, s, OCH_3), 3.93 (6H, s, OCH_3), 6.61 (2H, s, H-5 & H-6).

2,3,4,5-Tetramethoxyacetophenone (115)[336]

Acetic anhydride (17 g, 166.54 mmol) and $ZnCl_2$ (45 g, 333 mmol) were added to a solution of **114** (11 g, 56.00 mmol) in nitromethane (240 ml). The reaction mixture was stirred at 50 °C overnight under nitrogen. Water (150 ml) was added and the mixture was extracted with ethyl acetate (3 x 200 ml). The combined organic extracts were dried over $MgSO_4$ and the solvent removed under reduced pressure. Chromatography on silica gel (90:10 hexane/ethyl acetate, R_f 0.12) afforded **115** (9.8 g, 40.66 mmol, 77%) as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 2.57 (3H, s), 3.79 (3H, s), 3.84 (3H, s), 3.85 (3H s), 3.89 (3H, s), 7.00 (1H, s). MS *m/z* (API-ES): found 241 $(M+H)^+$ (100 %).

2-Hydroxy-3,4,5-tetramethoxyacetophenone (116)[335]

$AlCl_3$ (5.21 g, 39.12 mmol,) was added portion wise to a stirred solution of 115 (9.4 g, 39.12 mmol) in benzene (50 ml) at room temperature. Stirring was continued at 80 °C for 6h. After cooling to room temperature, the reaction mixture was poured into ice-water (140 ml) containing concd HCl (14 ml) and extracted with Et_2O (200 ml). The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. Chromatography on silica gel (90:10 hexane/ethyl acetate, R_f 0.12) afforded pure phenol **116** (4.8 g, 21.14 mmol, 54%) as a yellow solid, mp 70-72 °C. ^1H NMR ($CDCl_3$) δ 2.52 (3H, s), 3.78 (3H, s), 3.85 (3H, s), 3.92 (3H, s), 6.85 (1H, s), 11.42 (1H, s). MS *m/z* (API-ES): found 227 $(M+H)^+$ (100%).

6-Acetyl-2,3,4-trimethoxy-phenyl acetate (117)

DBU (0.148 g, 1.02 mmol) was added to a solution of **116** (0.100 g, 0.44 mmol) and acyl chloride (0.039 g, 0.51 mmol) in pyridine (1.1 ml). The resulting mixture was stirred at at 140

°C overnight. After cooling to room temperature, the mixture was poured into HCl (aq, 1 M, 10 ml) and extracted with ethyl acetate (2 x 10 ml). The organic extracts were collected, dried over $MgSO_4$ and the solvent removed under reduced pressure to afford pure **117** as a yellow oil. ^1H NMR ($CDCl_3$) δ 2.52 (3H, s), 2.80 (3H, s), 3.75 (3H, s), 3.83 (3H, s), 3.95 (3H, s), 6.89 (1H, s). MS *m/z* (API-ES): found 269 $(M+H)^+$ (100 %).

6,7,8-Trimethoxy-2-methylchromen-4-one (112)

DBU (0.148 g, 1.02 mmol) was added to a solution of **116** (0.100 g, 0.442 mmol) and acetic anhydride (0.052 g, 0.508 mmol) in pyridine (1.1 ml). The resulting mixture was stirred at at 140 °C overnight. After cooling to room temperature, the mixture was poured into HCl (aq, 1 M, 10 ml) and extracted with ethyl acetate (2 x 10 ml). The organic extracts were collected, dried over $MgSO_4$ and the solvent removed under reduced pressure. Chromatography on silica gel (80:20 DCM/ethyl acetate R_f 0.30) afforded pure chromenone **112** (0.012 g, 0.05 mmol, 11%) as a yellow solid.

Methyl -2-amino-3,4,5-trimethoxybenzoate (119)[337]

A solution of methyl-2-nitro-3,4,5-trimethoxybenzoate (**118**) (5.3 g, 19.54 mmol) and tin chloride dihydrate (22.7 g, 100.56 mmol) in ethanol (150 ml) was stirred at 80 °C for 4h under nitrogen. The solvent was removed under reduced pressure. The residue was treated with water (150 ml), made alkaline to pH 10 with NaOH, extracted with DCM (3 x 150 ml). The combined organic extracts were dried over $MgSO_4$ and the solvent evaporated *in vacuum*. Pure ester **119** was obtained as a yellow oil (4.6 g, 19.08 mmol, 98%) without further purification. ^1H NMR (250 MHz, $CDCl_3$) δ 3.74 (3H, s, OCH_3), 3.79 (6H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.59 (2H, bs, NH_2), 7.12 (1H, s, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.49 (OCH_3), 56.35 (OCH_3), 60.27 (OCH_3), 61.12 (OCH_3), 104.69 (C, Ar), 108.19 (CH, Ar), 139.53 (C, Ar), 140.27 (C, Ar), 143.43 (C, Ar), 147.33 (C, Ar), 168.05 (C=O).

2-Amino-3,4,5-trimethoxybenzoyc acid[338](120)

A solution of methyl-2-amino-3,4,5-trimethoxybenzoate (**119**) (3.8 g, 15.76 mmol) in 2-propanol (18 ml) was charged with 2.5 g (31.25 mmol) of 50% aqueous sodium hydroxide and 8 ml of water. The mixture was stirred at reflux for 4 h, and the solvent removed under reduced pressure. Pure acid **120** was obtained adjusting the pH to 4.5 with conc sulfuric acid and filtering the yellow solid The compound was used and characterized as obtained without further purification (4.27 g, 11.89 mmol, 75%), mp 136-138 °C (lit[412] 138-140 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 3.75 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 7.13 (1H, s, H-6).

6,7,8-Trimethoxy-2-methyl-benzo[d][1,3]oxazin-4-one (121)[413]

A solution of **120** (1.7 g, 7.49 mmol) in acetic anhydride (5 ml) was stirred for 1h at 110-120 °C, and the solvent was removed under reduced pressure. Pure oxazinone **121** was obtained after recrystallization from anhydrous ethyl acetate (1.76 g, 7.04 mmol, 94%), mp 129-131°C (lit[414] 134 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47 (3H, s, CH_3), 3.88 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 7.31 (1H, s, H-5).

6,7,8-Trimethoxy-2,3-dimethyl-3H-quinazolin-4-one (122a)

Methylamine (40% aq, 1.73 g 21.51 mmol) was added to a solution of **121** (3.6 g, 14.34 mmol) in THF (20 ml). The resulting mixture was stirred at room temperature for 20 minutes: the formation of a precipitate was observed. The solvent was removed under reduced pressure. The resulting residue was dissolved in glacial acetic acid (30 ml) and concd sulforic acid (2 drops) was added. The mixture was stirred for 1.15 h at 100 °C. The solvent was removed under reduced pressure. The residue was diluted with ethyl acetate (30 ml) and washed with a saturated aqueous solution of $NaHCO_3$ (3 x 20 ml). The organic extracts were dried over $MgSO_4$ and the solvent removed under reduced pressure. Chromatography on silica gel (ethyl acetate, R_f 0.60) afforded pure **122a** as a white solid (2.1 g, 7.95 mmol, 55%), mp 78-80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (3H, s, CH_3), 3.54 (3H, s, CH_3) 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 7.34 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.80 (CH_3), 31.11 (CH_3), 56.19 (OCH_3), 61.31 (OCH_3), 62.14 (OCH_3), 101.58 (CH, Ar), 116.25 (C, Ar), 137.27 (C, Ar), 147.16 (C, Ar), 147.41 (C, Ar), 152.24 (C, Ar), 153.61 (C=N), 161.79 (C=O). ν_{max} (solid)/(cm^{-1}) 1663 (st), 1597 (st), 1472 (st), 1427 (st), 1375 (st), 1198 (st), 1150 (st), 1098 (st). MS *m/z* (**API-ES**): found 265 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 256.1187 (M+H), calculated for $C_{13}H_{17}O_4N_2$ 265.1183.

3-Ethyl-6,7,8-trimethoxy-2-methyl-3H-quinazolin-4-one (122b)

This was obtained from **121** (2.02 g, 8.1 mmol) and ethylamine (70% aq., 0.78 g, 12.12 mmol) in a similar manner as described for preparation of **122a**. Chromatography on silica gel (ethyl acetate, R_f 0.68) afforded pure **122b** a white solid (1.5 g, 5.39 mmol, 67%), mp 84-86 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (3H, t, *J* 7.2 Hz, $NCH_2\underline{CH_3}$), 2.61 (3H, s, CH_3), 3.89 (3H, s, OCH_3) 3.94 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.10 (2H, q, *J* 7.2 Hz, $NC\underline{H_2}CH_3$), 7.35 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.74 ($NCH_2\underline{C}H_3$), 23.15 (CH_3), 39.63 ($N\underline{C}H_2CH_3$), 56.15 (OCH_3), 61.30 (OCH_3), 62.12 (OCH_3), 101.48 (CH, Ar), 116.60 (C, Ar), 137.32 (C, Ar), 147.18 (C, Ar), 147.39 (C, Ar), 151.72 (C, Ar), 152.30 (C=N), 161.35 (C=O). ν_{max} (solid)/(cm^{-1}) 1664 (st), 1591 (st), 1470 (st), 1396 (st), 1377 (st), 1095 (st). MS *m/z* (**API-ES**): found 279 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 279.1339 $(M+H)^+$, calculated for $C_{14}H_{19}O_4N_2$ 279.1338.

2-[2-(3-Chlorophenyl)vinyl]-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(123a).** A solution of **122a** (0.290 g, 1.1 mmol) and 3-chlorobenzaldehyde (0.232 g, 1.65 mmol) was stirred in presence of sodium methoxide (0.116 g, 2.2 mmol) in methanol (12 mL) at 80 °C for 24 h. After cooling to room temperature, pure product **123a** (0.121 g, 0.327 mmol, 30 %) was collected as a yellow precipitate by filtration and dried *in vacuo*, mp 145-147°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (3H, s, NCH_3), 3.90 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 7.03 (1H, d, *J* 15.6 Hz, CH), 7.20-7.27 (2H, m, ArH), 7.34-7.38 (2H, m, ArH), 7.52 (1H, s, ArH) 7.86 (1H, d, *J* 15.6 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.81 (NCH_3), 56.30 (OCH_3), 61.46 (OCH_3), 62.57 (OCH_3), 101.90 (CH, Ar), 116.74 (C, Ar), 120.43 (CH=CH), 126.16 (CH, Ar), 127.33 (CH, Ar), 129.53 (CH, Ar), 130.19 (CH, Ar), 134.93 (C, Ar), 137.36 (C, Ar), 137.46 (C, Ar), 138.84 (CH=CH), 147.55 (C, Ar), 147.60 (C, Ar), 149.64 (C, Ar), 152.74 (C=N), 161.80 (C=O),. ν_{max} (solid)/(cm^{-1}) 1663 (st), 1598 (st), 1484 (st), 1469 (st), 1425 (st), 1379 (st), 1198 (st), 1153 (st), 1098 (st). MS *m/z* (**API-ES**): found 387 (M ^{35}Cl+H)$^+$ (100%), 389 (M ^{37}Cl+H)$^+$ (35%). HRMS *m/z* (**API-ES**): found 387.1103 (M+H)$^+$, calculated for $C_{20}H_{20}N_2O_4Cl$ 387.1103.

2-[2-(4-Chlorophenyl)vinyl]-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(123b).** This was obtained as a yellow solid (0.363 g, 0.97 mmol, 84%) from **122a** (0.307 g, 1.16 mmol) and 4-chlorobenzaldehyde (0.180 g, 1.28 mmol) in a similar manner as described for preparation of **123a**, mp 154-156 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.76 (3H, s, CH_3), 3.97 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 4.15 (3H, s, OCH_3), 7.06 (1H, d, *J* 15.2 Hz, CH), 7.38 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.45 (1H, s, H-5), 7.54 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.95 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.74 (NCH_3), 56.25 (OCH_3), 61.43 (OCH_3), 62.53 (OCH_3), 101.86 (CH, Ar), 116.58 (C, Ar), 119.56 (CH), 128.92 (2 x CH, Ar), 129.15 (2 x CH, Ar), 133.99 (C, Ar), 135.43 (C, Ar), 137.47 (C, Ar), 138.94 (CH), 147.49 (C, Ar), 147.55 (C, Ar), 149.61 (C, Ar), 152.63 (C=N), 161.76 (C=O). ν_{max} (solid)/(cm^{-1}) 1663 (st), 1597 (st), 1544 (st), 1480 (st), 1468 (st), 1418 (st), 1373 (st), 1147 (st), 1087 (st), 1033 (st), 971 (st), 816 (st). MS *m/z* (**API-ES**): found 387 (M ^{35}Cl+H)$^+$ (100%), 389 (M ^{37}Cl+H)$^+$ (35%). HRMS *m/z* (**API-ES**) found 387.1100 (M+H)$^+$, calculated fo$C_{20}H_{20}N_2O_4Cl$ 387.1103.

2-[2-(2,4-Dichlorophenyl)vinyl]-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(123c).** This was obtained as a yellow solid (0.171 g, 0.41 mmol, 58%) from **122a** (0.187 g, 0.71 mmol) and 2.4-dichlorobenzaldehyde (0.149 g, 0.85 mmol) in a similar manner as described for preparation of **123a**, mp 183-185 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (3H, s, NCH_3), 4.09 (3H, s, OCH_3), 4.16 (3H, s, OCH_3), 4.29 (3H, s, OCH_3), 7.20 (1H, d, *J* 15.4 Hz, CH), 7.40 (1H, dd, *J* 2.0, 8.4 Hz, H-5'), 7.56 (1H, s, H-5), 7.58 (1H, d, *J* 2.0 Hz, H-2'), 7.74 (1H, d, *J* 8.4 Hz, H-6'), 8.41 (1H, d, *J* 15.4 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.83 (NCH_3), 56.29 (OCH_3), 61.48 (OCH_3), 62.59 (OCH_3), 101.81 (CH, Ar), 116.67 (C, Ar),

122.13 (CH=CH), 127.56 (CH, Ar), 128.18 (CH, Ar), 130.06 (CH, Ar), 132.36 (C, Ar), 135.23 (CH=CH), 135.61 (C, Ar), 137.42 (C, Ar), 147.54 (C, Ar), 147.62 (C, Ar), 149.25 (C, Ar), 152.80 (C=N), 161.75 (C=O). ν_{max} (solid)/(cm^{-1}) 1654 (st), 1594 (st), 1543 (st), 1481 (st), 1413 (st), 1382 (st), 1202 (st), 1150 (st), 1094 (st). MS *m/z* (**API-ES**): found 421 ($M^{35}Cl+H)^+$ (100%), 423 ($M^{37}Cl+H)^+$ (50%). HRMS *m/z* (**API-ES**) found 421.0722 $(M+H)^+$, calculated fo$C_{20}H_{19}N_2O_4Cl_2$ 421.0716.

2-[2-(2,6-Dichlorophenyl)vinyl]6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(122d).** This was prepared from **122a** (0.226 g, 0.85 mmol) and 2,4-dichlorobenzaldehyde (0.165 g, 0.94 mmol) in a similar manner as described for preparation of **123a**. Chromatography on silica gel (50:50 hexane/ethyl acetate, R_f 0.63) afforded pure **122d** as a yellow solid (0.224 g, 0.53 mmol, 62%), mp 175-177 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 3.68 (3H, s, NCH_3), 3.91 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.13 (3H, s, OCH_3), 7.13 (1H, t, *J* 8.0 Hz, H-4'), 7.32 (1H, d, *J* 15.8 Hz, CH), 7.33 (2H, d, *J* 8.0 Hz, H-3' & H-5'), 7.39 (1H, s, H-5), 8.05 (1H, d, *J* 15.8 H, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.85 (NCH_3), 56.29 (OCH_3), 61.50 (OCH_3), 62.65 (OCH_3), 101.78 (CH, Ar), 116.76 (C, Ar), 127.54 (CH=CH), 128.97 (2 x CH, Ar), 129.46 (CH, Ar), 132.69 (C, Ar), 133.70 (C, Ar), 134.97 (CH=CH), 137.49 (C, Ar), 147.46 (C, Ar), 147.69 (C, Ar), 149.20 (C, Ar), 152.75 (C=N), 161.83 (C=O). ν_{max} (solid)/(cm^{-1}) 2938 (st), 1667 (st), 1414 (st), 1370 (st), 1145 (st), 1095 (st), 1033 (st), 973 (st). MS *m/z* (**API-ES**): found 421 ($M^{35}Cl+H)^+$ (100%), 423 ($M^{37}Cl+H)^+$ (35%). HRMS *m/z* (**API-ES**): found 421.0713 $(M+H)^+$, calculated for $C_{20}H_{19}N_2O_4Cl_2$ 421.0716.

2-[2-(3,4-Dichlorophenyl)vinyl]-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(123e).** This was obtained as a yellow solid (0.051g, 0.123 mmol, 31%) from **122a** (0.102 g, 0.39 mmol) and 3,4-dichlorobenzaldehyde (0.081 g, 0.47 mmol) in a similar manner as described for preparation of **123a**, mp 145-147 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 3.82 (3H, s, NCH_3), 4.02 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 4.19 (3H, s, OCH_3), 7.14 (1H, d, *J* 15.3 Hz, CH), 7.44-7.53 (3H, m, ArH), 7.74 (1H, d, *J* 1.7 Hz, H-2'), 7.94 (1H, d, *J* 15.3 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.79 (NCH_3), 56.30 (OCH_3), 61.45 (OCH_3), 62.56 (OCH_3), 101.91 (CH, Ar), 116.70 (C, Ar), 120.79 (CH=CH), 126.93 (CH, Ar), 129.15 (CH, Ar), 130.90 (CH, Ar), 133.22 (C, Ar), 133.45 (C, Ar), 135.57 (C, Ar), 137.39 (C, Ar), 137.68 (CH=CH), 147.56 (C, Ar), 147.62 (C, Ar), 149.24 (C, Ar), 152.83 (C=N), 161.72 (C=O). ν_{max} (solid)/(cm^{-1}) 1662 (st), 1597 (st), 1545 (st), 1469 (st), 1372 (st), 1198 (st), 1147 (st), 1094 (st). MS *m/z* (**API-ES**): found 421 ($M^{35}Cl+H)^+$ (100%), 423 ($M^{37}Cl+H)^+$ (50%). HRMS *m/z* (**API-ES**): found 421.0720 $(M+H)^+$, calculated for $C_{20}H_{19}N_2O_4Cl_2$ 421.0716.

2-[2-(3,5-Dimethoxyphenyl)vinyl]-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(123f).** This was obtained as a yellow solid (0.176 g, 0.43 mmol, 75%) from **122a** (0.148 g, 0.56 mmol) and 3,5-dimethoxybenzaldehyde (0.122 g, 0.67 mmol) in a similar manner as

described for preparation of **123a**, mp 153-155 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (3H, s, NCH_3), 3.86 (6H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.17 (3H, s, OCH_3), 6.51 (1H, t, *J* 2.4 Hz, H-4'), 6.76 (2H, d, *J* 2.4 Hz, H-2' & H-6'), 7.08 (1H, d, *J* 15.2 Hz, CH), 7.45 (1H, s, H-5), 7.93 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.80 (NCH_3), 55.50 (2 x OCH_3), 56.28 (OCH_3), 61.45 (OCH_3), 62.57 (OCH_3), 101.66 (CH, Ar), 101.87 (CH, Ar), 105.85 (2 x CH, Ar), 116.59 (C, Ar), 119.63 (C̲H=CH), 137.47 (C, Ar), 137.58 (C, Ar), 140.44 (CH=C̲H), 147.52 (C, Ar), 147.56 (C, Ar), 149.79 (C, Ar), 152.59 (C, Ar), 161.08 (C=N), 161.86 (C=O). ν_{max} (solid)/(cm^{-1}) 1654 (st), 1593 (st), 1547 (st), 1457 (st), 1424 (st), 1413 (st), 1379 (st), 1342 (st), 1200 (st), 1146 (st), 1096 (st). MS *m/z* (**API-ES**): found 413 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 413.1705 $(M+H)^+$, calculated for $C_{22}H_{25}N_2O_6$ 413.1707.

2-[2-(2,5-Dimethoxyphenyl)vinyl]-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(123g).** This was obtained as a yellow solid (0.135 g, 0.32 mmol, 57%) from **122a** (0.151 g, 0.574 mmol) and 2,5-dimethoxybenzaldehyde in a similar manner as described for preparation of **123a**, mp 146-148 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (3H, s, NCH_3), 3.84 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.19 (3H, s, OCH_3), 6.91-6.96 (2H, m, H-3' & H-4'), 7.13 (1H, d, *J* 2.4 Hz, H-6'), 7.28 (1H, d, *J* 15.4 Hz, CH), 7.47 (1H, s, H-5), 8.20 (1H, d, *J* 15.4 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.82 (NCH_3), 55.87 (OCH_3), 56.16 (OCH_3), 56.27 (OCH_3), 61.48 (OCH_3), 62.57 (OCH_3), 101.76 (CH, Ar), 112.29 (CH, Ar), 114.43 (CH, Ar), 115.68 (C, Ar), 116.48 (CH, Ar), 120.58 (C̲H=CH), 125.25 (C, Ar), 136.14 (CH=C̲H), 147.43 (C, Ar), 147.51 (C, Ar), 150.49 (C, Ar), 150.80 (C, Ar), 152.38 (C, Ar), 152.74 (C, Ar), 153.53 (C=N), 162.00 (C=O). ν_{max} (solid)/(cm^{-1}) 1666 (st), 1590 (st), 1466 (st), 1418 (st), 1217 (st), 1145 (st), 1095 (st), 1039 (st). MS (**API-ES**) *m/z* found 413 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 413.1703 $(M+H)^+$, calculated for $C_{22}H_{25}N_2O_6$ 413.1707.

6,7,8-Trimethoxy-3-methyl-2-[2-(2,4,6-trimethoxyphenyl)vinyl]-3H-quinazolin-4-one **(123h).** This was obtained as a yellow solid (0.130 g, 0.29 mmol, 65%) from **122a** (0.120 g, 0.45 mmol) and 2,4,6-trimethoxybenzaldehyde (0.107 g, 0.54 mmol) in a similar manner as described for preparation of **123a**, 138-140 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75 (3H, s, NCH_3), 3.88 (3H, s, OCH_3), 3.94 (6H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.24 (3H, s, OCH_3), 6.18 (2H, s, H-3' & H-5'), 7.45 (1H, s, H-5), 7.53 (1H, d, *J* 15.5 Hz, CH), 8.46 (1H, d, *J* 15.5 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.60 (NCH_3), 55.39 (2 x OCH_3), 55.91 (OCH_3), 56.20 (OCH_3), 61.47 (OCH_3), 62.45 (OCH_3), 90.58 (2 x CH, Ar), 101.61 (CH, Ar), 106.99 (C, Ar), 116.07 (C, Ar), 118.71 (C̲H=CH), 131.51 (CH=C̲H), 138.08 (C, Ar), 147.20 (C, Ar), 147.33 (C, Ar), 151.79 (C, Ar), 152.00 (C, Ar), 160.84 (C, Ar), 162.16 (C=N), 162.28 (C=O). ν_{max} (solid)/(cm^{-1}) 1650 (st), 1599 (st), 1540 (st), 1416 (st), 1321 (st),

1149 (st), 1097 (st), 1036 (st). MS *m/z* (**API-ES**): found 443 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 443.1817 $(M+H)^+$, calculated for $C_{23}H_{27}N_2O_7$ 443.1813.

2-[2-(2,4-Dimethoxyphenyl)vinyl]-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(123i).** This was obtained as a yellow solid (0.155 g, 0.37 mmol, 56%) from **122a** (0.178 g, 0.67 mmol) and 2,4-dimethoxybenzaldehyde (0.135 g, 0.81 mmol) in a similar manner as described for preparation of **123a**, mp 164-166 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (3H, s, NCH_3), 3.77 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 6.43 (1H, d, *J* 2.4 Hz, H-3'), 6.47 (1H, dd, *J* 2.4, 8.4 Hz, H-5'), 7.12 (1H, d, *J* 15.2 Hz, CH), 7.35 (1H, s, H-5), 7.43 (1H, d, *J* 8.4 Hz, H-6'), 8.10 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.80 (NCH_3), 55.37 (OCH_3), 55.39 (OCH_3), 56.24 (OCH_3), 61.40 (OCH_3), 62.31 (OCH_3), 98.31 (CH, Ar), 101.46 (CH, Ar), 104.49 (CH, Ar), 116.54 (C, Ar), 117.63 (C, Ar), 121.31 (CH=CH), 130.33 (CH=CH), 132.33 (CH, Ar), 140.34 (C, Ar), 147.56 (C, Ar), 147.59 (C, Ar), 151.63 (C, Ar), 152.56 (C, Ar), 158.07 (C, Ar), 161.24 (C=N), 162.04 (C=O). ν_{max} (solid)/(cm^{-1}) 1658 (st), 1602 (st), 1585 (st), 1466 (st), 1420 (st), 1196 (st), 1146 (st), 1096 (st). MS *m/z* (**API-ES**): found 413 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 413.1706 $(M+H)^+$, calculated for $C_{22}H_{25}N_2O_6$ 413.1707.

6,7,8-Trimethoxy-3-methyl-2-[2-(2,3,4-trimethoxyphenyl)-vinyl]-3H-quinazolin-4-one **(123j).** This was obtained as a yellow solid (0.208 g, 0.47 mmol, 51%) from **122a** (0.245 g, 0.92 mmol) and 2,3,4-trimethoxybenzaldehyde (0.218 g, 1.11 mmol) in a similar manner as described for preparation of **123a**, mp 148-150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (3H, s, NCH_3), 3.84 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.90 (3H, , OCH_3), 3.90 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.12 (3H, s, OCH_3), 6.66 (1H, d, *J* 8.8 Hz, ArH), 7.14 (1H, d, *J* 15.4 Hz, CH), 7.24 (1H, d, *J* 8.8 Hz, ArH), 7.38 (1H, s, H-5), 8.06 (1H, d, *J* 15.4 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.77 (NCH_3), 56.09 (OCH_3), 56.26 (OCH_3), 60.99 (OCH_3), 61.22 (OCH_3), 61.48 (OCH_3), 62.49 (OCH_3), 101.79 (CH, Ar), 107.61 (CH), 116.39 (C, Ar), 118.47 (CH), 122.64 (C, Ar), 123.76 (CH=CH), 135.94 (CH=CH), 137.78 (C, Ar), 142.53 (C, Ar), 147.20 (C, Ar), 147.44 (C, Ar), 150.60 (C, Ar), 152.29 (C, Ar), 153.13 (C, Ar), 154.99 (C=N), 162.04 (C=O). ν_{max} (solid)/(cm^{-1}) 1658 (st), 1588 (st), 1463 (st), 1415 (st), 1092 (st). MS *m/z* (**API-ES**): found 443 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 443.1817 $(M+H)^+$, calculated foor $C_{23}H_{27}N_2O_7$ 443.1813.

6,7,8-Trimethoxy-3-methyl-2-[2-(3,4,5-trimethoxyphenyl)vinyl]-3H-quinazolin-4-one **(123k).** This was obtained as a yellow solid (0.0776 g, 0.17 mmol, 38%) from **122a** (0.121 g, 0.46 mmol) and 3,4,5-trimethoxybenzaldehyde (0.108 g, 0.55 mmol) in a similar manner as described for preparation of **123a**, mp 165-167 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (3H, s, NCH_3), 3.93 (3H, s, OCH_3), 3.96 (6H, s, OCH_3), 4.00 (3H, s, OCH_3), 4.06 (3H, s, OCH_3),

4.18 (3H, s, OCH_3), 6.86 (2H, s, H-2' & H-6'), 7.01 (1H, d, *J* 15.2 Hz, CH), 7.49 (1H, s, H-5), 7.96 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.85 (NCH_3), 56.27 (2 x OCH_3), 61.05 (OCH_3), 61.45 (OCH_3), 62.58 (OCH_3), 101.88 (CH, Ar), 104.98 (2 x CH, Ar), 116.51 (C, Ar), 118.33 (CH=CH), 131.15 (C, Ar), 137.63 (C, Ar), 139.66 (C, Ar), 140.54 (CH=CH), 147.48 (C, Ar), 147.58 (C, Ar), 149.90 (C, Ar), 152.53 (C, Ar), 153.50 (C=N), 161.90 (C=O). ν_{max} (solid)/(cm^{-1}) 1665 (st), 1577 (st), 1471 (st), 1417 (st), 1376 (st), 1331 (st), 1148 (st), 1126 (st), 1096 (st). MS *m/z* (**API-ES**): found 443 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 443.1817 $(M+H)^+$, calculated for $C_{23}H_{27}N_2O_7$ 443.1813.

2-[2-(3,5-Dimethoxyphenyl)vinyl]-3-ethyl-6,7,8-trimethoxy-3H-quinazolin-4-one **(123l).** This was obtained as a yellow solid (0.084 g, 0.19 mmol, 57%) from **122b** (0.096 g, 0.346 mmol) and 3,5-dimethoxybenzaldehyde (0.086 g, 0.52 mmol) in a similar manner as described for preparation of **123a**; reaction time 48 h, mp 168-17- °C. ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (3H, t, *J* 7.2 Hz, NCH_2CH_3), 3.86 (6H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.17 (3H, s, OCH_3), 4.34 (2H, q, *J* 7.2 Hz, NCH_2CH_3), 6.51 (1H, t, *J* 2.0 Hz, H-4'), 6.76 (2H, d, *J* 2.0 Hz, H-2' & H-6'), 7.06 (1H, d, *J* 15.2 Hz, CH), 7.47 (1H, s, H-5), 7.97 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.39 (NCH_2CH_3), 38.69 (NCH_2CH_3), 55.48 (2 x OCH_3), 56.23 (OCH_3), 61.45 (OCH_3), 62.57 (OCH_3), 101.32 (CH, Ar), 101.74 (CH, Ar), 105.87 (2 x CH, Ar), 116.76 (C, Ar), 119.44 (CH=CH), 137.56 (C, Ar), 137.61 (C, Ar), 140.54 (CH=CH), 147.48 (C, Ar), 147.52 (C, Ar), 149.29 (C, Ar), 152.52 (C, Ar), 161.04 (C=N), 161.41 (C=O). ν_{max} (solid)/(cm^{-1}) 1668 (st), 1589 (st), 1471 (st), 1414 (st), 1144 (st). MS *m/z* (**API-ES**): found 427 $(M+H)^+$ (100%). HRMS *m/z* (API-ES): found 427.1888 $(M+H)^+$ (100%), calculated for $C_{23}H_{27}N_2O_6$ 427.1869.

2-[2-(2,5-Dimethoxyphenyl-vinyl]-3-ethyl-6,7,8-trimethoxy-3H-quinazolin-4-one **(123m).** This was obtained as a yellow solid (0.094 g, 0.22 mmol, 64%) from **122b** (0.096 g, 0.34 mmol) and 2,5-dimethoxybenzaldehyde (0.086 g, 0.52 mmol) in a similar manner as described for preparation of **123a**; reaction time 48 h, mp 170-172 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 1.36 (3H, t, *J* 7.2 Hz, NCH_2CH_3), 3.76 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 4.25 (2H, q, *J* 7.2 Hz, NCH_2CH_3), 6.82-6.84 (2H, m, ArH), 7.01-7.04 (1H, m ArH), 7.24 (1H, d, *J* 15.2 Hz, CH), 7.38 (1H, s, ArH), 8.12 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.28 (NCH_2CH_3), 38.78 (NCH_2CH_3), 55.87 (OCH_3), 56.14 (OCH_3), 56.24 (OCH_3), 61.49 (OCH_3), 62.58 (OCH_3), 101.67 (CH, Ar), 112.27 (CH, Ar), 114.89 (CH, Ar), 115.30 (CH, Ar), 116.68 (C, Ar), 120.61 (C, Ar), 125.39 (CH=CH), 136.34 (CH=CH), 137.78 (C, Ar), 147.42 (C, Ar), 147.49 (C, Ar), 150.01 (C, Ar), 152.34 (C, Ar), 152.76 (C, Ar), 153.51 (C=N), 161.57 (C=O). ν_{max} (solid)/(cm^{-1}) 1661 (st), 1586 (st), 1463 (st), 1423 (st), 1373 (st), 1218 (st), 1145

(st), 1097 (st), 1042 (st). MS *m/z* (**API-ES**) found 427 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 427.1664 $(M+H)^+$, calculated for $C_{23}H_{27}N_2O_6$ 427.1664.

2-[2-(2,4-Dimethoxyphenyl)vinyl]-3-ethyl-6,7,8-trimethoxy-3H-quinazolin-4-one **(123n).** This was obtained as a yellow solid (0.035 g, 0.0821 mmol, 12%) from **122b** (0.188 g, 0.681 mmol) and 2,4-dimethoxybenzaldehyde (0.169 g, 1.02 mmol) in a similar manner as described for preparation of **123a**; reaction time 48 h, mp 159-161 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (3H, t, *J* 7.2 Hz, $NCH_2C\underline{H}_3$), 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.20 (3H, s, OCH_3), 4.34 (2H, q, *J* 7.2 Hz, $NC\underline{H}_2CH_3$), 6.52 (1H, t, *J* 2.4 Hz, H-3'), 6.56 (1H, dd, *J* 2.4, 8.6 Hz, H-5'), 7.25 (1H, d, *J* 15.2 Hz, CH), 7.46 (1H, s, H-5), 7.51 (1H, d, *J* 8.6 Hz, H-6'), 8.20 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.20 ($NCH_2\underline{C}H_3$), 38.70 ($N\underline{C}H_2CH_3$), 55.51 (OCH_3), 55.60 (OCH_3), 56.22 (OCH_3), 61.48 (OCH_3), 62.53 (OCH_3), 98.61 (CH, Ar), 101.64 (CH, Ar), 105.11 (CH, Ar), 116.47 (C, Ar), 117.63 (<u>C</u>H=CH), 117.93 (C, Ar), 131.04 (CH, Ar), 136.50 (CH=<u>C</u>H), 137.94 (C, Ar), 147.38 (C, Ar), 150.55 (C, Ar), 152.07 (C, Ar), 159.69 (C, Ar), 161.69 (C=N), 161.99 (C=O). ν_{max} (solid)/(cm^{-1}) 1668 (st), 1589 (st), 1463 (st), 1425 (st), 1198 (st), 1147 (st), 1095 (st). MS *m/z* (**API-ES**): found 427 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 427.1866 $(M+H)^+$ (100%), calculated for $C_{23}H_{27}N_2O_6$ 427.1869.

3-Ethyl-6,7,8-trimethoxy-2-[2-(2,4,5-trimethoxyphenyl)vinyl]-3H-quinazolin-4-one **(123o).** This was obtained as a yellow solid (0.076 g, 0.17 mmol, 49%) from **122b** (0.095 g, 0.34 mmol) and 2,4,5-trimethoxybenzaldehyde (0.100 g, 0.51 mmol) in a similar manner as described for preparation of **123a**; reaction time 48 h, mp 181-183 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, *J* 7.2 Hz, $NCH_2C\underline{H}_3$), 3.84 (3H, s), 3.85 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 4.26 (2H, q, *J* 7.2 Hz, $NC\underline{H}_2CH_3$), 6.54 (1H, s, ArH), 6.98 (1H, s, ArH), 7.15 (1H, d, *J* 15.2 Hz, CH), 7.38 (1H, s, ArH), 7.62 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.21 ($NCH_2\underline{C}H_3$), 38.74 ($N\underline{C}H_2CH_3$), 56.10 (OCH_3), 56.23 (OCH_3), 56.39 (OCH_3), 56.73 (OCH_3), 61.48 (OCH_3), 62.54 (OCH_3), 97.03 (CH, Ar), 101.68 (CH, Ar), 112.82 (CH, Ar), 116.48 (C, Ar), 117.86 (<u>C</u>H=CH), 136.36 (CH=<u>C</u>H), 137.92 (C, Ar), 143.13 (C, Ar), 147.37 (C, Ar), 147.42 (C, Ar), 150.51 (C, Ar), 151.23 (C, Ar), 152.12 (C, Ar), 153.78 (C=N), 161.6 (C=O). ν_{max} (solid)/(cm^{-1}) 1643 (st), 1536 (st), 1465 (st), 1293 (st), 1210 (st), 1027 (st). MS *m/z* (**API-ES**): found 452 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 452.1967 $(M+H)^+$, calculated for $C_{24}H_{29}N_2O_7$ 452.1969.

3-Ethyl-6,7,8-trimethoxy-2-[2-(2,4,6-trimethoxyphenyl)vinyl]-3H-quinazolin-4-one **(123p).** This was obtained as a yellow solid (0.127 g, 0.28 mmol, 84%) from **122b** (0.092 g, 0.33 mmol) and 2,4,6-trimethoxybenzaldehyde (0.098 g, 0.50 mmol) in a similar manner as

described for preparation of **123a**; reaction time 48 h, mp 188-190 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (3H, t, *J* 7.2 Hz, $NCH_2C\underline{H}_3$), 3.87 (3H, s, OCH_3), 3.92 (6H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 4.23 (3H, s, OCH_3), 4.33 (2H, q, *J* 7.2 Hz, $NC\underline{H}_2CH_3$), 6.18 (2H, s, H-3' & H-5'), 7.44 (1H, s, H-5), 7.56 (1H, d, *J* 15.6 Hz, CH), 8.46 (1H, d, *J* 15.6 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.06 ($NCH_2\underline{C}H_3$), 38.63 ($N\underline{C}H_2CH_3$), 55.40 (OCH_3), 55.89 (2 x OCH_3), 56.17 (OCH_3), 61.48 (OCH_3), 62.47 (OCH_3), 90.59 (2 x CH, Ar), 101.51 (CH, Ar), 107.08 (C, Ar), 116.27 (C, Ar), 118.74 ($\underline{C}H$=CH), 131.46 (CH=$\underline{C}H$), 138.12 (C, Ar), 147.18 (C, Ar), 147.31 (C, Ar), 151.46 (C, Ar), 151.75 (C, Ar), 160.77 (C, Ar), 161.85 (C=N), 162.07 (C=O). ν_{max} (solid)/(cm^{-1}) 1650 (st), 1600 (st), 1539 (st), 1453 (st), 1321 (st), 1147 (st), 1101 (st), 1040 (st). MS *m/z* (**API-ES**): found 457 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 457.2006 $(M+H)^+$ (100%), calculated for $C_{24}H_{29}N_2O_7$ 457.1975.

2-[2-(3,4-Dichlorophenyl)vinyl]-3-ethyl-6,7,8-trimethoxy-3H-quinazolin-4-one **(123q).** This was obtained as a yellow solid (0.043 g, 0.09 mmol, 20%) from **122b** (0.138 g, 0.49 mmol) and 3,4-dichlorobenzaldehyde (0.130 g, 0.75 mmol) in a similar manner as described for preparation of **123a**; reaction time 48 h, mp 157-159 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, *J* 7.0 Hz, $NCH_2C\underline{H}_3$), 3.91 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 4.27 (2H, q, *J* 7.0 Hz, $NC\underline{H}_2CH_3$), 7.00 (1H, d, *J* 15.2 Hz, CH), 7.36-747 (3H, m, ArH), 7.62 (1H, d, *J* 1.6 Hz, H-2'), 7.87 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ14.48 ($NCH_2\underline{C}H_3$), 38.74 ($N\underline{C}H_2CH_3$), 56.27 (OCH_3), 61.47 (OCH_3), 62.57 (OCH_3), 101.81 (CH, Ar), 116.87 (C, Ar), 120.55 ($\underline{C}H$=CH), 126.89 (CH, Ar), 129.11 (C, Ar), 133.20 (CH, Ar), 133.39 (CH, Ar), 135.65, 137.42 (CH=$\underline{C}H$), 137.92 (C, Ar), 147.53 (C, Ar), 147.10 (C, Ar), 148.80 (C, Ar), 152.79 (C, Ar), 161.30 (C=N), 167.80 (C=O). ν_{max} (solid)/(cm^{-1}) 1665 (st), 1597 (st), 1542 (st), 1479 (st), 1388 (st), 1146 (st), 1096 (st). MS *m/z* (APCI-MS): 435 $[M+ H]^+$ (100%), MS *m/z* $[M+H]^+$ (100 %) 435.0878. %). MS *m/z* (**API-ES**): found 435 (M ^{35}Cl+H)$^+$ (100%), 437 (M^{37}Cl+H)$^+$ (70%). HRMS *m/z* (**API-ES**): found 435.0878 $(M+H)^+$, calculated for $C_{20}H_{20}N_2O_4Cl_2$ 435.0873.

2-[2-(4-Chlorophenyl)vinyl]-3-ethyl-6,7,8-trimethoxy-3H-quinazolin-4-one **(123r).** This was obtained as a yellow solid (0.106 g, 0.26 mmol, 57%) from **122b** (0.130 g, 0.47 mmol) and 4-chlorobenzaldehyde (0.098 g, 0.70 mmol) in a similar manner as described for preparation of **123a**; reaction time 48 h, mp 145-147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (3H, t, *J* 7.1 Hz, $NCH_2C\underline{H}_3$), 4.02 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.17 (3H, s, OCH_3), 4.35 (2H, q, *J* 7.1 Hz, $NC\underline{H}_2CH_3$), 7.08 (1H, d, *J* 15.2 Hz, CH), 7.38 (2H, d, *J* 9.4 Hz, 2 x CH, Ar), 7.48 (1H, s, H-5), 7.58 (2H, d, *J* 9.4 Hz, 2 x CH, Ar), 8.01 (1H, d, *J* 15.2 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.42 ($NCH_2\underline{C}H_3$), 38.70 ($N\underline{C}H_2CH_3$), 56.25 (OCH_3), 61.46 (OCH_3), 62.55 (OCH_3), 101.77 (CH, Ar), 116.78 (C, Ar), 119.35 ($\underline{C}H$=CH), 128.90 (2 x CH, Ar) 129.17 (2 x CH, Ar), 134.09 (C, Ar), 135.38 (C, Ar), 137.55 (C, Ar), 139.16

(CH=CH), 147.48 (C, Ar), 147.56 (C, Ar), 149.16 (C, Ar), 152.62 (C=N), 161.37 (C=O). ν_{max} (solid)/(cm^{-1}) 1664 (st), 1469 (st), 1367 (st), 1146 (st), 1085 (st), 1035 (st). MS *m/z* (**API-ES**): found 401 (M ^{35}Cl+H)$^+$ (100%), 403 (M ^{37}Cl+H)$^+$ (35%). HRMS *m/z* (**API-ES**): found 401.1356 (M+ H)$^+$ (100%), calculated for $C_{21}H_{22}N_2O_4$ 401.1268.

3,5-Bis-(methoxymethoxy)benzaldehyde (125)

NaH (60% suspension in mineral oil, 0.072g, 1.794 mmol) was added to a solution of 3,5-dihydroxybenzaldehyde (**124**) (0.116 g, 0.815 mmol) in DMF (5 ml) at room temperature under Ar at 0 °C. After stirring for 30 min and MOMchoride (0.144 g, 1.794 mmol) was added portion wise. The reaction mixture was stirred at romm temperature overnight and H_2O (5 ml) was added. The aqueous phase was extracted with ethyl acetate (3 x 5 ml). The combined organic extracts were washed with brine (5ml), dried over Na_2SO_4 and the solvent removed under reduced pressure. Chromatography on silica gel (60:40 DCM/ethyl acetate, R_f 0.54) afforded the 3,5-bis-(methoxymethoxy)benzaldehyde (**125**) (0.180 g, 0.800 mmol, 98%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) 3.98 (6H, s, OCH_3), 5.20 (4H, s, CH_2), 6.65 (1H, t, *J* 2.0 Hz, H-4), 7.03 (2H, d, *J* 2.0 Hz, H-2 & H-6), 9.79 (1H, s, CHO).

***2-[2-(3,5-Bis-(methoxymethoxy)phenyl)-vinyl]-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one* (112s).** This was obtained as a yellow solid (0.192 g, 0.406 mmol, 34%) from **122a** (0.313 g, 1.18 mmol) and **125** (0.291 g, 1.28 mmol) in a similar manner as described for preparation of **123a**, mp 200-202 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.50 (6H, s, 2 x OCH_3), 3.75 (3H, s, NCH_3), 3.97 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 4.14 (3H, s, OCH_3), 5.19 (4H, s, 2 x CH_2), 6.77 (1H, s, H-4'), 6.94 (2H, d. *J* 1.6 Hz, H-2' & H6'), 7.05 (1H, d, *J* 15.4 Hz, CH), 7.45 (1H, s, H-5), 7.90 (1H, d, *J* 15.4 Hz, CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.04 (NCH_3), 56.38 (2 x OCH_3), 56.49 (OCH_3), 61.64 (OCH_3), 62.79 (OCH_3), 94.76 (2 x CH_2), 102.99 (CH, Ar), 106.30 (CH, Ar), 109.34 (2 x CH, Ar), 116.83 (C, Ar), 120.06 (CH=CH), 137.81 (CH=CH), 137.89 (C, Ar), 140.35 (C, Ar), 147.79 (C, Ar), 149.95 (C, Ar), 152.83 (C, Ar), 158.50 (C=N), 162.07 (C=O). ν_{max} (solid)/(cm^{-1}) 1649 (st), 1597 (st), 1471 (st), 1374 (st), 1143 (st), 1036 (st). MS *m/z* (**API-ES**): found 473 (M+ H)$^+$ (100%). HRMS (**API-ES**) *m/z* found 473.1923 (M+H)$^+$, calculated for $C_{24}H_{29}N_2O_8$ 473.1924.

2-[2-(3,5-Dihydroxyphenyl)vinyl]-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one (123t)

Compound **123s** (0.163 g, 0.34 mmol) was suspended in methanol (5 ml), and HCl (aq, 4M, 1.4 ml) was added. The reaction mixture was stirred at 80 °C for 45 minutes. After cooling to room temperature, the solvent was removed under reduced pressure. The resulting solid was was washed with water (10 ml), filtered, and dried under vaccum. Pure **123t** was obtained as a yellow solid (0.120 g, 0.31 mmol, 90%) without further purification, mp 232-234 °C, ^{1}H NMR (400 MHz, CD_3OD) δ 3.77 (3H, s, NCH_3), 4.02 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 4.13 (3H, s, OCH_3), 6.44 (1H, t, *J* 1.8 Hz, H-4'), 6.72 (2H, d. *J* 1.8 Hz, H-2' & H-6'), 7.21

(1H, d, *J* 15.8 Hz, CH), 7.53 (1H, s, H-5), 7.65 (1H, d, *J* 15.8 Hz, CH). ^{13}C (DMS0-d_6) δ 31.10 (NCH_3), 56.65 (OCH_3), 61.60 (OCH_3), 62.82 (OCH_3), 102.20 (CH, Ar), 104.98 (CH, Ar) 106.81 (2 x CH, Ar), 116.62 (C, Ar), 120.13 (CH=CH), 137.09 (C, Ar), 137.58 (C, Ar), 140.85 (CH=CH), 147.62 (C, Ar), 147.76 (C, Ar), 151.09 (C, Ar), 152.77 (C, Ar), 159.41 (C=N), 161.26 (C=O). ν_{max} (solid)/(cm^{-1}) 3521 (st), 3052 (st), 1695 (st), 1626 (st), 1591 (st), 1476 (st), 1389 (st), 1161 (st). MS *m/z* (**API-ES**): found 383 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**) found 383.1248 $(M-H)^-$, calculated for $C_{20}H_{19}N_2O_6$ 383.1243.

2-[2-(3,5-Bis(methoxymethoxy)phenyl)vinyl]-3-ethyl-6,7,8-trimethoxy-3H-quinazolin-4-one **(123u).** This was obtained as a yellow solid (0.127 g, 0.26 mmol, 31%) from **122b** (0.215 g, 0.83 mmol) and **125** (0.175 g, 0.77 mmol) in a similar manner as described for preparation of **123a**, mp 187-189 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (3H, t, *J* 7.4 Hz, NCH_2CH_3), 3.51 (6H, s, 2 x OCH_3), 3.98 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 4.16 (3H, s, OCH_3), 4.32 (2H, q, *J* 7.4 Hz, NCH_2CH_3), 5.21 (4H, s, 2 x CH_2), 6.79 (1H, t, *J* 2.4 Hz, H-4'), 6.95 (2H, d. *J* 2.4 Hz, H-2' & H-6'), 7.05 (1H, d, *J* 15.4 Hz, CH), 7.46 (1H, s, H-5), 7.94 (1H, d, *J* 15.4 Hz, CH). ^{13}C NMR ($CDCl_3$) δ 14.58 (NCH_2CH_3), 38.92 (NCH_2CH_3), 56.39 (2 x OCH_3), 56.46 (OCH_3), 61.65 (OCH_3), 62.79 (OCH_3), 102.32 (CH, Ar), 104.91 (CH, Ar) 106.81 (2 x CH, Ar), 116.62 (C, Ar), 120.13 (CH=CH), 137.09 (C, Ar), 137.58 (C, Ar), 140.76 (CH=CH), 147.69 (C, Ar), 147.72 (C, Ar), 151.013 (C, Ar), 152.75 (C, Ar), 159.45 (C=N), 161.29 (C=O). ν_{max} (solid)/(cm^{-1}) 1653 (st), 1591 (st), 1467 (st), 1146 (st), 1029 (st). MS *m/z* (**API-ES**): found 487 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 487.2088 $(M+H)^+$, Calculated for $C_{26}H_{30}N_2O_8$ 487.2080.

2-[2-(3,5-Dihydroxyphenyl)vinyl]-3-ethyl-6,7,8-trimethoxy-3H-quinazolin-4-one **(123v).** This was obtained as a yellow solid (0.037 g, 0.085 mmol, 95%) from **123u** (0.047 g, 0.09 mmol) in a similar manner as described for preparation of **123t**, mp 245-247 °C, ^{1}H NMR (400 MHz, CD_3OD) δ 1.38 (3H, t, *J* 7.2 Hz, NCH_2CH_3), 3.94 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 4.34 (2H, q, *J* 7.2 Hz, NCH_2CH_3), 6.31 (1H, t, *J* 2.0 Hz, H-4'), 6.60 (2H, d. *J* 2.0 Hz, H-2' & H-6'), 7.14 (1H, d, *J* 15.2 Hz, CH), 7.42 (1H, s, H-5), 7.77 (1H, d, *J* 15.2 Hz, CH). ^{13}C (DMS0-d_6) δ 14.59 (NCH_2CH_3), 38.90 (NCH_2CH_3), 56.63 (OCH_3), 61.61 (OCH_3), 62.82 (OCH_3), 102.20 (CH, Ar), 104.98 (CH, Ar) 106.81 (2 x CH, Ar), 116.62 (C, Ar), 120.13 (CH=CH), 137.09 (C, Ar), 137.58 (C, Ar), 140.85 (CH=CH), 147.62 (C, Ar), 147.76 (C, Ar), 151.09 (C, Ar), 152.77 (C, Ar), 159.41 (C=N), 161.26 (C=O). δ . ν_{max} (solid)/(cm^{-1}) 3462 (st), 3245 (st), 1635 (st), 1598 (st), 1469 (st), 1280 (st), 1146 (st). MS *m/z* (**API-ES**): found 397 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 397.1405 $(M-H)^-$, calculated for $C_{21}H_{21}N_2O_6$ 397.1400.

6,7,8-Trimethoxy-2-(3-methoxyphenyl)benzo[d][1,3]oxazin-4-one (126b)

3-Methoxybenzoyl chloride (0.195 g, 1.14 mmol) was added dropwise to a solution of **120** (0.130 g, 0.57 mmol) in pyridine (4 ml) at 0 °C. The reaction mixture was stirred at room temperature for 1h and then poured into ice-water. The precipitate was filtered, washed with water (5 ml), and dried under vacuum. Pure **126b** was obtained as a white solid (0.175 g, 0.510 mmol, 89%), without further purification, mp 115-117 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.06 (3H,s, OCH_3), 4.17 (3H, s, OCH_3), 7.10 (1H, ddd, *J* 1.4, 2.7, 8.3 Hz, ArH), 7.41 (1H, t, *J* 8.3 Hz, ArH), 7.44 (1H, s, H-5), 7.81 (1H, t, *J* 1.4 Hz, ArH), 7.91 (1H, dt, *J* 1.4, 8.3 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.75 (OCH_3), 56.65 (OCH_3), 61.74 (OCH_3), 63.02 (OCH_3), 104.28 (CH, Ar), 112.62 (CH, Ar), 112.79 (CH, Ar), 118.95 (CH, Ar), 120.84 (CH, Ar), 129.96 (C, Ar), 131.99 (C, Ar), 136.62 (C, Ar), 148.11 (C, Ar), 149.64 (C, Ar), 153.65 (C, Ar), 155.17 (C, Ar), 159.73 (C=N), 160.06 (C=O). ν_{max} (solid)/(cm^{-1}) 2941 (md), 1760 (st), 1614 (st), 1482 (st), 1464 (st), 1363 (st), 1281 (st), 1111 (st). MS *m/z* (**API-ES**): found 344 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 344.1154 $(M+H)^+$, calculated for $C_{18}H_{18}NO_6$ 344.1134.

6,7,8-Trimethoxy-2-phenyl-benzo[d][1,3]oxazin-4-one **(126a).** This was obtained as yellow solid (0.849 g, 2.71 mmol, 78%) from **120** (0.800 g, 3.52 mmol) and benzoyl chloride (0.992 g, 7.04 mmol) in a similar manner as described for preparation of **126b**, mp 160-162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 4.18 (3H, s, OCH_3), 7.45 (1H, s, H-5), 7.49-7.59 (3H, m, ArH), 8.31 (2H, dd, *J* 1.8, 6.8 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.64 (OCH_3), 61.74 (OCH_3), 63.01 (OCH_3), 104.26 (CH, Ar), 112.61 (C, Ar), 128.26 (2 x CH, Ar), 128.92 (2 x CH, Ar), 130.66 (C, Ar), 132.51 (CH, Ar), 136.68 (C, Ar), 148.11 (C, Ar), 149.65 (C, Ar), 153.62 (C, Ar), 155.34 (C=N), 159.75 (C=O). ν_{max} (solid)/(cm^{-1}) 1746 (st), 1615 (md), 1473 (st), 1427 (md), 1358 (st), 1289 (md), 1109 (st), 1013 (md), 846 (st), 764 (st), 700 (st), 685 (st). MS *m/z* (**API-ES**): found 314 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 314.1053 $(M+H)^+$, calculated for $C_{17}H_{16}NO_5$ 314.1028.

6,7,8-Trimethoxy-2-(4-methoxyphenyl)benzo[d][1,3]oxazin-4-one **(126c).** This was prepared from **120** (0.800 g, 3.52 mmol) and 3-methoxybezoyl chloride (1.20 g, 7.04 mmol) in a similar manner as described for preparation of **126b**. Chromatography on silica gel (6:4 hexane:ethyl acetate, R_f 0.35) afforded pure **126c** as white solid (0.551 g, 1.60 mmol, 46%), mp 160-162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.16 (3H, s, OCH_3), 7.00 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 7.42 (1H, s, H-5), 8.26 (2H, d, *J* 9.2 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 54.90 (OCH_3), 55.43 (OCH_3), 60.71 (OCH_3), 62.45 (OCH_3), 101.49 (CH, Ar), 114.56 (2 x CH, Ar), 116.45 (C, Ar), 124.90 (C, Ar), 128.76 (2 x CH, Ar), 140.17 (C, Ar), 147.95 (C, Ar), 148.34 (C, Ar), 148.89 (C, Ar), 152.80 (C, Ar), 162.67 (C=N), 163.91 (C=O). ν_{max} (solid)/(cm^{-1}) 3285

(md), 1745 (st), 1667 (st), 1545 (st), 1417 (st), 1354 (st), 1265 (st), 1074 (st). MS *m/z* (**API-ES**): found 344 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 344.1153 $(M+H)^+$, calculated for $C_{18}H_{18}NO_6$ 344.1134.

6,7,8-Trimethoxy-2-(3,4-dimethoxy-phenyl)-benzo[d][1,3]oxazin-4-one **(126d)**. This was obtained as yellow solid (0.198 g, 0.53 mmol, 72%) from **120** (0.167 g, 0.73 mmol) and 3,4-dimethoxybezoyl chloride (0.295 g, 1.47 mmol) in a similar manner as described for preparation of **126b**, mp 202-206 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 4.16 (3H, s, OCH_3), 6.96 (1H, d, *J* 8.8 Hz, H-5'), 7.42 (1H, s, H-5), 7.79 (1H, d, *J* 2.0 Hz, H-2'), 7.95 (1H, d, *J* 2.0, 8.8 Hz, H-6'). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.30 (OCH_3), 56.34 (OCH_3), 56.62 (OCH_3), 61.74 (OCH_3), 62.91 (OCH_3), 104.28 (CH, Ar), 110.54 (CH, Ar), 110.98 (CH, Ar), 112.26 (C, Ar), 122.34 (CH, Ar), 123.18 (C, Ar), 137.05 (C, Ar), 147.83 (C, Ar), 149.24 (C, Ar), 149.69 (C, Ar), 152.96 (C, Ar), 153.27 (C, Ar), 155.38 (C=N), 159.93 (C=O). ν_{max} (solid)/(cm^{-1}) 3383 (md), 1736 (st), 1613 (st), 1512 (st) , 1472 (st), 1450 (st), 1417 (st), 1360 (st), 1303 (st), 1267 (st), 1112 (st), 1009 (st), 763 (st). MS *m/z* (**API-ES**): found 374 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 374.1268 $(M+H)^+$, calculated for $C_{19}H_{20}NO_7$ 374.1240.

Benzyl 3-benzyloxy-4-methoxybenzoate (129)[415]

Potassium carbonate (2.71 g, 19.62 mmol) and benzyl bromide (2.34 g, 13.73 mmol) were added to an ice-cooled solution of 3-hydroxy-4-methoxy benzoic acid (**128**) (1.1 g, 6.54 mmol) in dry DMF (12 ml) under Ar. The reaction mixture was stirred overnight at room temperature. Water (15 ml) was added and the resulting mixture extracted with DCM (3 x 15 ml). The combined organic extracts were dried (Na_2SO_4) and the solvent removed under reduced pressure. Chromatography on silica gel (8:2 hexane/ethyl acetate, R_f 0.40) afforded pure **129** as white solid (2.0 g, 5.74 mmol, 88 %), mp 66- 88 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (3H, s, OCH_3), 5.17 (2H, s, CH_2), 5.31 (2H, s, ArH), 6.89 (1H, d, *J* 8.6 Hz, H-5), 7.25-7.40 (10H, m, ArH), 7.62 (1H, d, *J* 2.0 Hz, H-2), 7.72 (1H, dd, *J* 2.0, 8.6 Hz, H-6).

3-Benzyl-4-methoxybenzoic acid (130)[416]

Sodium hydroxide (aq, 5N, 20 ml) was added to a solution of **129** (1.6 g, 4.6 mmol) in 30 ml isopropanol. The reaction mixture was stirred at 100 °C for 3 h. Isopropanol was removed under reduced pressure. The remaining aqueous solution was acidified with conc. HCl and extracted with DCM (3 x 30 ml). The combined organic extracts were dried over Na_2SO_4 and the solvent removed under reduced pressure. Chromatography on silica gel (6:4 hexane:ethyl acetate, R_f 0.31) gave pure **130** as white solid (1.1 g, 4.26 mmol, 92%), mp 170-172 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.95 (3H, s, OCH_3), 5.19 (2H, s, CH_2), 6.94 (1H, d, *J* 7.9 Hz, ArH), 7.37-7.48 (3H, m, ArH), 7.48 (2H, d, *J* 1.6 Hz, ArH), 7.65 (1H, d, *J* 1.9 Hz, H-2), 7.77 (1H, dd, *J* 1.9, 8.6 Hz, H-6).

2-(3-Benzyloxy-4-methoxyphenyl)-6,7,8-trimethoxybenzo[d][1,3]oxazin-4-one (126e)

Oxalyl chloride (0.284 g, 1.95 mmol) was added drop wise to a solution of **120** (0.421 g, 1.63 mmol) and dry DMF (2 drops) in dry DCM (15 ml) at 0 °C under Ar. The reaction mixture was stirred at 0 °C for 2 h. The solvent was removed under reduced pressure to give a yellow solid. The crude material was dissolved in pyridine (7 ml) and **120** (0.185 g, 0.81 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 1h. Water (10 ml) was added and the product was extracted with DCM (3 x 10 ml). The combined organic extracts were dried over Na_2SO_4 and the solvent removed under reduced pressure. Recrystalization from ethyl acetate afforded pure **126e** as a yellow solid (0.229 g, 0.51 mmol, 62 %), mp 145-147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 5.27 (2H, s, CH_2), 6.98 (1H, d, *J* 8.4 Hz, Ar-H), 7.30-7.41 (4H, m, ArH), 7.50-7.52 (2H, m, ArH), 7.85 (1H, d, *J* 1.9 Hz, ArH), 7.93 (1H, dd, *J* 1.9, 8.4 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.43 (OCH_3), 56.75 (OCH_3), 61.70 (OCH_3), 62.79 (OCH_3), 71.38 (CH_2), 101.68 (CH, Ar), 111.75 (CH, Ar), 112.69 (CH, Ar), 116.99 (C, Ar), 120.30 (CH, Ar), 125.43 (C, Ar), 127.35 (2 x CH, Ar), 128.32 (CH, Ar), 129.01 (2 x CH, Ar), 135.03 (C, Ar), 139.81 (C, Ar), 148.31 (C, Ar), 147.88 (C, Ar), 148.23 (C, Ar), 148.75 (C, Ar), 151.80 (C, Ar), 152.67 (C=N), 162.81 (C=O). ν_{max} (solid)/(cm^{-1}) 3132 (md), 1645 (st), 1580 (st), 1034 (st), 730 (st). MS *m/z* (**API-ES**): found 450 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 450.1571 $(M+H)^+$, calculated for $C_{25}H_{24}NO_7$ 450.1553.

6,7,8-Trimethoxy-2-phenyl-3H-quinazolin-4-one (127a)

A mixture of **126a** (0.119 g, 0.38 mmol) and aqueous ammonium hydroxde (0.041 g, 1.17 mmol) in pyridine (2 ml) was heated in the microwave reactor at 140 °C for 30 min. After cooling to room temperature, the reaction mixture was poured in HCl (aq, 1M, 10 ml) and extracted with ethyl acetate (2 x 15 ml). The organic extracts were collected, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Chromatography on silica gel with (5:5 hexanes/ethyl acetate, R_f 0.32) afforded pure **127a** (0.065 g, 0.20 mmol, 55%) as white solid, mp 231-233 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.18 (3H, s, OCH_3), 7.50 (1H, s, H-5), 7.56 (3H, m, ArH), 8.05-8.08 (2H, m, Ar-H), 9.62 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.48 (OCH_3), 61.71 (OCH_3), 62.82 (OCH_3), 101.65 (CH, Ar), 117.14 (C, Ar), 127.14 (2 x CH, Ar), 129.24 (2 x CH, Ar), 131.68 (CH, Ar), 132.99 (C, Ar), 139.60 (C, Ar), 148.45 (C, Ar), 148.49 (C, Ar), 149.14 (C, Ar), 153.07 (C=N), 162.86 (C=O). ν_{max} (solid)/(cm^{-1}) 2929 (md), 1661 (st), 1464 (st), 1126 (st), 1074 (md), 684 (st). MS *m/z* (**API-ES**): found 313 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 313.1195 $(M+H)^+$, calculated for $C_{17}H_{17}N_2O_4$ 313.1188.

6,7,8-Trimethoxy-2-(3-methoxyphenyl)-3H-quinazolin-4-one **(127b).** This was prepared from **126b** (0.152 g, 0.44 mmol) and aqueous ammonium hydroxide (0.079 g, 2.26 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel (4:6 hexanes/ethyl acetate, R_f 0.60) afforded **127b** as a white solid (0.103 g, 0.30 mmol, 68%), mp 125-127 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 4.19 (3H, s, OCH_3), 7.09 (1H, dd, *J* 1.9, 8.3 Hz, ArH), 7.45 (1H, t, *J* 8.3 Hz, ArH), 7.51 (1H, s, H-5), 7.75 (1H, d, *J* 8.3 Hz, ArH), 7.82 (1H, t, *J* 1.9 Hz, ArH), 10.93 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.75 (OCH_3), 56.40 (OCH_3), 61.71 (OCH_3), 62.82 (OCH_3), 101.56 (CH, Ar), 112.52 (CH, Ar), 117.17 (C, Ar), 117.63 (CH, Ar), 119.71 (CH, Ar), 130.19 (CH, Ar), 134.51 (C, Ar), 139.75 (C, Ar), 148.44 (C, Ar), 149.15 (C, Ar), 153.01 (C, Ar), 160.31 (C=N), 163.35 (C=O). ν_{max} (solid)/(cm^{-1}) 3145 (st), 1632b (st), 1446 (st), 1089 (st), 611 (st). MS *m/z* (**API-ES**): found 343 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 343.1301 $(M+H)^+$, calculated for $C_{18}H_{19}N_2O_5$ 343.1294.

6,7,8-Trimethoxy-2-(4-methoxyphenyl)-3H-quinazolin-4-one **(127c)** This was prepared from **116c** (0.063 g, 0.21 mmol) and aqueous ammonium hydroxide (0.037 g, 1.08 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel (3:7 hexanes/ethyl acetate, R_f 0.42) afforded **127c** as a white solid (0.050 g, 0.14 mmol, 69%), mp 191–193 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.18 (3H, s, OCH_3), 7.04 (2H, d, *J* 9.0 Hz, 2 x CH, ArH), 7.48 (1H, s, H-5), 8.09 (2H, d, *J* 9.0 Hz, 2 x CH, ArH), 10.17 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.67 (OCH_3), 56.43 (OCH_3), 61.70 (OCH_3), 62.75 (OCH_3), 101.56 (CH, Ar), 114.45 (2 x CH, Ar), 116.77 (C, Ar), 125.49 (C, Ar), 128.98 (2 x CH, Ar), 140.05 (C, Ar), 148.22 (C, Ar), 148.44 (C, Ar), 149.13 (C, Ar), 152.60 (C, Ar), 162.40 (C=N), 163.44 (C=O). ν_{max} (solid)/(cm^{-1}) 3280 (st), 1631 (st), 1414 (st), 1123 (st), 730 (st). MS *m/z* (**API-ES**): found 343 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 343.1295 $(M+H)^+$, calculated for $C_{18}H_{19}N_2O_5$ 343.1294.

6,7,8-Trimethoxy-2-(3,4-dimethoxyphenyl)-3H-quinazolin-4-one **(127d).** This was prepared from **126d** (0.180 g, 0.48 mmol) and aqueous ammonium hydroxide (0.086 g, 2.46 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel (ethyl acetate, R_f 0.44) afforded **127d** (0.103 g, 0.27 mmol, 57%) as a white solid, mp 206-208 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.19 (3H, s, OCH_3), 7.00 (1H, d, *J* 8.4 Hz, H-5'), 7.47 (1H, s, H-5), 7.68 (1H, dd, *J* 2.0, 8.4 Hz, H-6'), 7.77 (1H, d, *J* 2.0 Hz, H-2'), 10.99 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.27 (OCH_3), 56.42 (2 x OCH_3), 61.70 (OCH_3), 62.72 (OCH_3), 101.41 (CH, Ar), 110.39 (CH, Ar), 111.22 (CH, Ar), 116.67 (C, Ar), 120.95 (CH, Ar), 125.65 (C, Ar), 139.94 (C, Ar), 148.16 (C, Ar), 148.48 (C, Ar), 149.37 (C, Ar), 149.42 (C, Ar), 152.07 (C, Ar), 152.71 (C=N), 163.79 (C=O). ν_{max} (solid)/(cm^{-1}) 3163 (md), 1644 (st), 1457

(st), 1122 (st), 1026 (st), 855 (st). MS *m/z* (**API-ES**): found 373 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 373.1412 $(M+H)^+$, calculated for $C_{19}H_{21}N_2O_6$ 373.1400.

6,7,8-Trimethoxy-2-phenyl-3-methyl-3H-quinazolin-4-one **(127e).** This was prepared from **126a** (0.206 g, 0.66 mmol) and methylamine (40% aq, 0.031 g, 1.01 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel (5:5, hexane/ethyl acetate, R_f 0.23) afforded pure **127e** as a white solid (0.101 g, 0.31 mmol, 47%), mp 131-133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.50 (3H, s, NCH_3), 3.99 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 7.48-7.52 (4H, m, ArH), 7.56-7.60 (2H, m, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.66 (NCH_3), 56.51 (OCH_3), 61.60 (OCH_3), 62.50 (OCH_3), 101.72 (CH, Ar), 116.86 (C, Ar), 128.68 (2 x CH, Ar), 128.82 (2 x CH, Ar), 130.07 (CH, Ar), 135.90 (C, Ar), 137.71 (C, Ar), 147.80 (C, Ar), 148.10 (C, Ar), 153.13 (C, Ar), 153.90 (C=N), 162.56 (C=O). ν_{max} (solid)/(cm^{-1}) 1670 (st), 1557 (st), 1471 (st), 1413 (st), 1374 (st), 1037 (st), 1034 (st). MS *m/z* (**API-ES**): found 327 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 327.1348 $(M+H)^+$, calculated for $C_{18}H_{19}N_2O_4$ 327.1345.

6,7,8-Trimethoxy-2-(3-methoxyphenyl)-3-methyl-3H-quinazolin-4-one **(127f).** This was prepared from **126b** (0.128 g, 0.373 mmol) and methylamine (40% aq, 0.035 g, 1.14 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel (3:7 with hexane/ethyl acetate, R_f 0.40) afforded **127f** as a white solid (0.089 g, 0.25 mmol, 67%), mp 139-141 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.50 (3H, s, NCH_3), 3.86 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 7.03 (1H, ddd, *J* 0.8, 2.8, 7.7 Hz, ArH), 7.10-7.14 (2H, m, ArH), 7.41 (1H, t, *J* 7.7 Hz, ArH), 7.50 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.59 (NCH_3), 55.68 (OCH_3), 56.51 (OCH_3), 61.60 (OCH_3), 62.51 (OCH_3), 101.72 (CH, Ar), 114.42 (CH, Ar), 115.62 (CH, Ar), 116.92 (C, Ar), 120.88 (CH, Ar), 129.97 (CH, Ar), 137.07 (C, Ar), 137.66 (C, Ar), 147.79 (C, Ar), 148.18 (C, Ar), 153.68 (C, Ar), 153.68 (C, Ar), 159.92 (C=N), 162.55 (C=O). ν_{max} (solid)/(cm^{-1}) 1663 (st), 1593 (st), 1578 (st), 1563 (st), 1478 (st), 1420 (st), 1379 (st), 1251 (st), 1140 (st), 1094 (st), 1026 (st). MS *m/z* (**API-ES**): found 357 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 357.1463 $(M+H)^+$, calculated for $C_{19}H_{21}N_2O_5$ 357.145.

6,7,8-Trimethoxy-2-(4-methoxyphenyl)-3-methyl-3H-quinazolin-4-one **(127g).** This was prepared from **112c** (0.135 g, 0.39 mmol) and methylamine (40% aq, 0.037 g, 1.20 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel performed using the FlashMaster purification station (60:40 hexane/ethyl acetate) afforded pure **127g** as a white solid (0.029 g, 0.081 mmol, 21%), mp 150-152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.54 (3H, s, NCH_3), 3.88 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 7.01 (2H, d, *J* 9.2 Hz, 2 x CH, ArH), 7.48 (1H, s, H-5), 7.55 (2H, d, *J* 9.2 Hz, 2 x CH, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.74 (NCH_3), 55.97 (OCH_3), 56.38

(OCH_3), 61.64 (OCH_3), 62.60 (OCH_3), 101.58 (CH, Ar), 114.60 (2 x CH, Ar), 116.76 (C, Ar), 125.61 (C, Ar), 128.72 (2 x CH, Ar), 140.01 (C, Ar), 148.11 (C, Ar), 148.43 (C, Ar), 149.16 (C, Ar), 152.63 (C, Ar), 162.38 (C=N), 163.65 (C=O). ν_{max} (solid)/(cm^{-1}) 2984 (md), 1663 (st), 1583 (st), 1515 (st), 1465 (st), 1414 (st), 1377 (st), 1350 (st), 1253 (st), 1177 (st), 1137 (st), 1090 (st). MS *m/z* (**API-ES**): found 357 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 357.1463 $(M+H)^+$, calculated for $C_{19}H_{21}N_2O_5$ 357.1450.

6,7,8-Trimethoxy-2-(3,4-dimethoxyphenyl)-3-methyl-3H-quinazolin-4-one **(127h).** This was prepared from **126d** (0.189 g, 0.20 mmol) and methylamine (40% aq, 0.048 g, 1.55 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel (3:7 hexane/ethyl acetate, R_f 0.43) afforded pure **127h** as a white solid (0.109 g, 0.28 mmol, 56%), mp 166-168 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.47 (3H, s, NCH_3), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 6.90 (1H, d, *J* 8.0 Hz, H-5'), 7.05 (1H, d, *J* 2.0 Hz, H-2'), 7.09 (1H, dd, *J* 2.0, 8.0 Hz, H-6'), 7.42 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.86 (NCH_3), 56.27 (OCH_3), 56.33 (OCH_3), 56.49 (OCH_3), 61.59 (OCH_3), 62.46 (OCH_3), 101.70 (CH, Ar), 111.25 (CH, Ar), 112.04 (CH, Ar), 116.75 (C, Ar), 121.74 (CH, Ar), 128.47 (C, Ar), 137.68 (C, Ar), 147.76 (C, Ar), 148.00 (C, Ar), 149.20 (C, Ar), 150.66 (C, Ar), 153.03 (C, Ar), 153.73 (C=N), 162.72 (C=O). ν_{max} (solid)/(cm^{-1}) 1659 (st), 1603 (md), 1518 (st), 1468 (st), 1372 (st), 1261 (st), 1240 (st), 1143 (st), 109 (st), 1064 (st). MS *m/z* (**API-ES**): found 387 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 387.1562 $(M+H)^+$, calculated for $C_{20}H_{23}N_2O_6$ 387.1556.

6,7,8-Trimethoxy-2-phenyl-3-ethyl-3H-quinazolin-4-one **(127i).** This was prepared from **126a** (0.192 g, 0.63 mmol) ethyl amine (70% aq, 0.085 g, 1.89 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel performed using the FlashMaster purification station (70:30 hexane/ethyl acetate) afforded pure **127i** as a white solid (0.027 g, 0.081 mmol, 13%), mp 128-130 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (3H, t, *J* 7.2 Hz, $NCH_2C\underline{H_3}$), 3.99 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.02-4.07 (2H, m, $NC\underline{H_2}CH_3$), 7.48-7.55 (6H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.28 ($NCH_2C\underline{H_3}$), 56.48 (OCH_3), 61.47 (OCH_3), 62.50 ($NC\underline{H_2}CH_3$), 62.66 (OCH_3), 101.39 (CH, Ar), 117.26 (C, Ar), 127.23 (2 x CH, Ar), 129.36 (2 x CH, Ar), 131.81 (CH, Ar), 132.80 (C, Ar), 139.50 (C, Ar), 148.35 (C, Ar), 148.55 (C, Ar), 149.23 (C, Ar), 153.15 (C=N), 162.67 (C=O). ν_{max} (solid)/(cm^{-1}) 3014 (md), 1654 (st), 1464 (st), 1134 (st), 1123(md), 750 (st). MS *m/z* (**API-ES**): found 341 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 341.1525 $(M+H)^+$, calculated for $C_{19}H_{21}N_2O_4$ 341.1501.

6,7,8-Trimethoxy-2-(3-methoxyphenyl)-3-ethyl-3H-quinazolin-4-one **(127j).** This was prepared from **126b** (0.183 g, 0.53 mmol) and ethylamine (70% aq, 0.073 g, 1.63 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel performed

using the FlashMaster purification station (70:30 hexane/ethyl acetate) afforded **127j** as a white solid in (0.037 g, 0.100 mmol, 19%), mp 98-100 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (3H, t, *J* 7.2 Hz, $NCH_2C\underline{H_3}$), 3.86 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.03-4.07 (2H, m, $NC\underline{H_2}CH_3$), 7.03 (1H, ddd, *J* 0.8, 2.8, 7.7 Hz, ArH), 7.10-7.14 (2H, m, ArH), 7.41 (1H, t, *J* 7.7 Hz, ArH), 7.50 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.32 ($NCH_2C\underline{H_3}$), 54.80 (OCH_3), 56.32 (OCH_3), 61.88 (OCH_3), 62.34 ($NC\underline{H_2}CH_3$), 62.49 (OCH_3), 101.55 (CH, Ar), 114.38 (CH, Ar), 115.65 (CH, Ar), 116.59 (C, Ar), 120.76 (CH, Ar), 129.66 (CH, Ar), 137.13 (C, Ar), 137.71 (C, Ar), 147.67 (C, Ar), 148.10 (C, Ar), 153.64 (C, Ar), 153.71 (C, Ar), 156.00 (C=N), 162.31 (C=O). ν_{max} (solid)/(cm^{-1}) 1621 (st), 1581 (st), 1565 (st), 1431 (st), 1251 (st), 1123 (st), 1075 (st). MS *m/z* (**API-ES**): found 371 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**) found 371.1634 $(M+H)^+$, calculated for $C_{20}H_{23}N_2O_5$ 371.1607

6,7,8-Trimethoxy-2-(4-methoxyphenyl)-3-ethyl-3H-quinazolin-4-one **(127k).** This was prepared from **126c** (0.171 g, 0.49 mmol) and e ethylamine (70% aq, 0.069 g, 1.53 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel performed using a FlashMaster purification station (65:35 hexane/ethyl acetate) afforded pure **127k** as a white solid (0.012 g, 0.032 mmol, 6%), mp 127-129 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (3H, t, *J* 7.2 Hz, $NCH_2C\underline{H_3}$), 3.88 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.08 (2H, q, *J* 7.2 Hz, $NC\underline{H_2}CH_3$), 7.00 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 7.48 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 7.49 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.30 ($NCH_2C\underline{H_3}$), 41.49 (OCH_3), 55.64 (OCH_3), 56.46 (OCH_3), 61.59 (OCH_3), 62.45 ($NC\underline{H_2}CH_3$), 101.70 (CH, Ar), 114.10 (2 x CH, Ar), 117.19 (C, Ar), 128.64 (C, Ar), 129.94 (2 x CH, Ar), 137.66 (C, Ar), 147.75 (C, Ar), 148. 00 (C, Ar), 152.98 (C, Ar), 153.88 (C, Ar), 160.75 (C=N), 162.03 (C=O). ν_{max} (solid)/(cm^{-1}) 1666 (st), 1608 (md), 1479 (st), 1251 (st), 1097 (st), 844 (st). MS *m/z* (**API-ES**): found 371 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 371.1618 $(M+H)^+$, calculated for $C_{20}H_{23}N_2O_5$ 371.1607.

6,7,8-Trimethoxy-2-(3,4-dimethoxyphenyl)-3-ethyl-3H-quinazolin-4-one **(127l).** This was prepared from **126d** (0.014 g, 0.30 mmol) and ethylamine (70% aq, 0.042 g, 0.93 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel (3:7 hexane/ethyl acetate, R_f 0.42) afforded pure **127l** as a white solid (0.034 g, 0.085 mmol, 28%), mp 118-121 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (3H, t, *J* 7.6 Hz, $NCH_2C\underline{H_3}$), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 4.07 (2H, q, *J* 7.6 Hz, $NC\underline{H_2}CH_3$), 6.96 (1H, d, *J* 8.2 Hz, H-5'), 7.05 (1H, d, *J* 2.0 Hz. H-2'), 7.11 (1H, dd, *J* 2.0, 8.0 Hz, H-6'), 7.49 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.45 ($NCH_2C\underline{H_3}$), 56.31 (OCH_3), 56.37 (OCH_3), 56.53 (OCH_3), 61.70 (OCH_3), 62.43 ($NC\underline{H_2}CH_3$), 62.51 (OCH_3), 101.47 (CH, Ar), 111.15 (CH, Ar), 112.09 (CH, Ar), 116.70 (C, Ar), 121.60 (CH, Ar), 128.53 (C, Ar), 137.72 (C, Ar), 147.74 (C, Ar), 148.09 (C,

Ar), 149.43 (C, Ar), 150.66 (C, Ar), 153.10 (C, Ar), 154.02 (C=N), 162.65 (C=O). ν_{max} (solid)/(cm^{-1}) 1666 (st), 1543 (st), 1450 (st), 1112 (st), 1054 (st), 1001 (st). MS *m/z* (**API-ES**): found 401 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 401.1720 $(M+H)^+$, calculated for $C_{21}H_{25}N_2O_6$ 401.1713.

6,7,8-Trimethoxy-2-phenyl-3-propyl-3H-quinazolin-4-one **(127m)**. This was prepared from **126a** (0.186 g, 0.59 mmol) and isopropylamine (0.108 g, 1.83 mmol) in a similar manner as described for preparation of **117a**. Chromatography on silica gel performed using a FlashMaster purification station (70:30 hexane/ethyl acetate) afforded afforded pure **127m** as a white solid in (0.009 g, 0.025 mmol, 4%), mp 91-93 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71 (3H, t, *J* 7.6 Hz, $NCH_2CH_2C\underline{H_3}$), 1.62 (2H, six, *J* 7.6 Hz, $NCH_2C\underline{H_2}CH_3$), 3.93-3.98 (2H, m, $NC\underline{H_2}CH_2CH_3$), 3.99 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), (3H, s, OCH_3), 7.47-7.54 (6H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.37 ($NCH_2CH_2\underline{C}H_3$), 22.32 ($NCH_2\underline{C}H_2CH_3$), 47.71 ($N\underline{C}H_2CH_2CH_3$), 56.46 ($OCH_3$), 61.59 ($OCH_3$), 62.46 ($OCH_3$), 101.76 (CH, Ar), 117.25 (C, Ar), 128.48 (2 x CH, Ar), 128.70 (2 x CH, Ar), 129.78 (CH, Ar), 136.14 (C, Ar), 137.58 (C, Ar), 147.78 (C, Ar), 148.07 (C, Ar), 153.10 (C, Ar), 154.04 (C=N), 162.01 (C=O). ν_{max} (solid)/(cm^{-1}) 2987 (st), 1667 (st), 1543 (st), 1435 (st), 1234 (st), 1034 (md). MS *m/z* (**API-ES**): found 355 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 355.1698 $(M+H)^+$, calculated for $C_{20}H_{23}N_2O_4$ 355.1658.

6,7,8-Trimethoxy-2-(3-methoxyphenyl)-3-propyl-3H-quinazolin-4-one **(127n).** This was prepared from **126b** (0.197 g, 0.57 mmol) and isopropylamine (0.104 g, 1.76 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel performed using the FlashMaster purification station (70:30 hexane/ethyl acetate) afforded pure **127n** as a white solid (0.077 g, 0.20 mmol, 34%), mp 131-133°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 (3H, t, *J* 7.6 Hz, $NCH_2CH_2C\underline{H_3}$), 1.64 (2H, sextuplet, *J* 7.6 Hz, $NCH_2C\underline{H_2}CH_3$), 3.86 (3H, s, OCH_3s), 3.93-3.97 (2H, m, $NC\underline{H_2}CH_2CH_3$), 4.01 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 4.06 (3H, s, OCH_3), 7.02-7.09 (3H, m, ArH), 7.39 (1H, t, *J* 8.0 Hz, ArH), 7.50 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.09 ($NCH_2CH_2\underline{C}H_3$), 21.98 ($NCH_2\underline{C}H_2CH_3$), 47.67 ($N\underline{C}H_2CH_2CH_3$), 54.34 ($OCH_3$), 56.54 ($OCH_3$), 61.91 ($OCH_3$), 62.32 ($NC\underline{H_2}CH_3$), 62.54 ($OCH_3$), 101.62 (CH, Ar), 114.23 (CH, Ar), 115.76 (CH, Ar), 116.76 (C, Ar), 120.68 (CH, Ar), 129.75 (CH, Ar), 137.19 (C, Ar), 137.77 (C, Ar), 147.76 (C, Ar), 148.14 (C, Ar), 153.59 (C, Ar), 153.82 (C, Ar), 156.06 (C=N), 162.54 (C=O). ν_{max} (solid)/(cm^{-1}) 2989 (st), 1664 (st), 1543 (st), 1436 (st), 1178 (st). MS *m/z* (**API-ES**): found 385 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 385.1791 $(M+H)^+$, calculated for $C_{21}H_{25}N_2O_5$ 385.1763.

6,7,8-Trimethoxy-2-(4-methoxyphenyl)-3-propyl-3H-quinazolin-4-one **(127o).** This was prepared from **126c** (0.139 g, 0.40 mmol) and isopropylamine (0.073 g, 1.24 mmol) in a similar manner as described for preparation of **117a**. Chromatography on silica gel performed

using the FlashMaster purification station (65:35 hexane/ethyl acetate) afforded pure **127o** as a white solid (0.007 g, 0.018 mmol, 5%), mp 134-136 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (3H, t, *J* 7.6 Hz, $NCH_2CH_2C\underline{H_3}$), 1.66 (2H, sextuplet, *J* 7.6 Hz, $NCH_2C\underline{H_2}CH_3$), 3.88 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 3.98-4.02 (2H, m, $NC\underline{H_2}CH_2CH_3$), 4.06 (3H, s, OCH_3), 6.99 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.47 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.48 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.18 ($NCH_2CH_2\underline{C}H_3$), 22.11 ($NCH_2\underline{C}H_2CH_3$), 47.56 ($N\underline{C}H_2CH_2CH_3$), 55.41 ($OCH_3$), 56.14 ($OCH_3$), 61.37 ($OCH_3$), 62.24 ($OCH_3$), 101.55 (CH, Ar), 113.85 (2 x CH, Ar), 116.92 (C, Ar), 128.46 (C, Ar), 129.83 (2 x CH, Ar), 137.42 (C, Ar), 147.53 (C, Ar), 147.79 (C, Ar), 152.76 (C, Ar), 153.74 (C, Ar), 160.51 (C=N), 162.00 (C=O). ν_{max} (solid)/(cm^{-1}) 3109 (md), 1665 (st), 1489 (st), 1234(st), 1045 (st). MS *m/z* (**API-ES**): found 385 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 385.1763 $(M+H)^+$, calculated for $C_{21}H_{25}N_2O_5$ 385.1763.

6,7,8-Trimethoxy-2-(3,4-dimethoxy-phenyl)-3-propyl-3H-quinazolin-4-one **(127p)** This was prepared from **126d** (0.167 g, 0.44 mmol) and isopropylamine (0.081 g, 1.37 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel (4:6 hexane/ethyl acetate, R_f 0.42) afforded pure **127p** as a white solid (0.026 g, 0.062 mmol, 14%), mp 119-121 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (3H, t, *J* 7.8 Hz, $NCH_2CH_2C\underline{H_3}$), 1.63 (2H, sextuplet, *J* 7.8 Hz, $NCH_2C\underline{H_2}CH_3$), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 3.99-4.02 (2H, m, $NC\underline{H_2}CH_2CH_3$), 4.02 (3H, s, 3H, s, OCH_3), 4.07 (3H, s, OCH_3), 6.96 (1H, d, *J* 8.2 Hz, H-5'), 7.04 (1H, d, *J* 2.0 Hz, H-2'), 7.10 (1H, dd, *J* 2.0, 8.2 Hz, H-6'), 7.49 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.01 ($NCH_2CH_2\underline{C}H_3$), 21.89 ($NCH_2\underline{C}H_2CH_3$), 47.78 ($N\underline{C}H_2CH_2CH_3$), 56.35 ($OCH_3$), 56.41 ($OCH_3$), 56.65 ($OCH_3$), 61.43 ($OCH_3$), 62.68 ($OCH_3$), 101.73 (CH, Ar), 111.17 (CH, Ar), 112.13 (CH, Ar), 116.45 (C, Ar), 122.03 (CH, Ar), 128.42 (C, Ar), 137.89 (C, Ar), 148.02 (C, Ar), 148.15 (C, Ar), 150.15 (C, Ar), 150.58 (C, Ar), 153.17 (C, Ar), 153.94 (C=N), 162.76 (C=O). ν_{max} (solid)/(cm^{-1}) 1645 (st), 1543 (st), 1487 (st), (st), 1113 (st), 1076 (st). MS *m/z* (**API-ES**): found 415 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 415.1868 $(M+H)^+$, calculated for $C_{22}H_{27}N_2O_6$ 415.1869.

2-(3-Benzyloxy-4-methoxyphenyl)-6,7,8-trimethoxy-3H-quinazolin-4-one **(127q)**. This was prepared from **126e** (0.129 g, 0.28 mmol) and aqueous ammonium hydroxide (0.030 g, 0.86 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica gel (7:3, DCM/ethyl acetate R_f 0.38) afforded pure **127q** as a white solid (0.087 g, 0.19 mmol, 68%), mp 195-197 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 4.05 (3H, s, OCH_3), 4.12 (3H, s, OCH_3), 5.31 (2H, s, CH_2), 7.01 (1H, d, *J* 8.5 Hz, H-5'), 7.31 (1H, d, *J* 7.2 Hz, ArH), 7.36 (2H, t, *J* 7.2 Hz, Ar-H), 7.45 (1H, s, H-5), 7.56 (2H, d, *J* 7.2 Hz, ArH), 7.66 (1H, dd, *J* 1.6, 8.5 Hz, H-6'), 7.82 (1H, d, *J* 1.6 Hz, H-2'), 10.36 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.37 (OCH_3), 56.41 (OCH_3), 61.70 (OCH_3), 62.75

(OCH_3), 71.34 (CH_2), 101.67 (CH, Ar), 111.66 (CH, Ar), 112.78 (CH, Ar), 116.85 (C, Ar), 120.27 (CH, Ar), 125.55 (C, Ar), 127.55 (2 x CH, Ar), 128.28 (CH, Ar), 128.87 (2 x CH, Ar), 136.84 (C, Ar), 139.79 (C, Ar), 148.28 (C, Ar), 148.45 (C, Ar), 148.68 (C, Ar), 148.77 (C, Ar), 152.76 (C, Ar), 152.85 (C=N), 162.77 (C=O). ν_{max} (solid)/(cm^{-1}) 3102 (st), 1654 (st), 1501 (st), 1123 (st), 1022 (st). MS *m/z* (**API-ES**): found 449 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 449.1723 $(M+H)^+$, calculated for $C_{25}H_{24}N_2O_6$ 449.1713.

2-(3-Hydroxy-4-methoxyphenyl)-6,7,8-trimethoxy-3H-quinazolin-4-one (127r)
A mixture of **127q** (0.071 g, 0.158 mmol) and 10% Pd/C (0,0071 g) in THF (5 mL) and methanol (5 mL) was stirred at room temperature under H_2 for 4 h. The solution was filtered through a celite bed and the solvent removed under reduced pressure to give pure **127r** as a white solid (0.054 g, 0.150 mmol, 95 %), mp 234-236 °C. ^1H NMR (400 MHz, CD_3OD) δ 3.94 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 7.07 (1H, d, *J* 8.4 Hz, H-5'), 7.46 (1H, s, H-5), 7.56 (1H, dd, *J* 2.4, 8.4 Hz, H-6'), 7.61 (1H, d, *J* 2.4 Hz, H-2'). ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.37 (OCH_3), 56.60 (OCH_3), 61.57 (OCH_3), 62.75 (OCH_3), 101.83 (CH, Ar), 112.18 (CH, Ar), 115.30 (CH, Ar), 117.44 (C, Ar), 119.58 (CH, Ar), 126.01 (C, Ar), 139.29 (C, Ar), 147.16 (C, Ar), 147.83 (C, Ar), 148.43 (C, Ar), 150.07 (C, Ar), 151.18 (C, Ar), 152.46 (C=N), 162.40 (C=O). ν_{max} (solid)/(cm^{-1}) 1665 (st), 1570 (st), 1469 (st), 1421 (st), 1290 (st), 1210 (st), 1129 (st), 1076 (st), 1032 (st), 866 (st), 800 (st). MS *m/z* (**API-ES**): found 359 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 359.1231 $(M+H)^+$, calculated for $C_{18}H_{19}N_2O_6$ 359.1243.

2-(3-Benzyloxy-4-methoxyphenyl)-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(127s).** This was prepared from **126e** (0.222 g, 0.49 mmol) and methylamine (40% aq, 0.046 g, 1.48 mmol) in a similar manner as described for preparation of **127a**. Chromatography on silica (9:1 DCM/ethyl acetate, R_f 0.46) afforded **127s** as a white solid (0.169 g, 0.36 mmol, 74%), mp 150-152 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 3.33 (3H, s, NCH_3), 3.97 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 5.22 (2H, s, CH_2), 6.99 (1H, d, *J* 8.0 Hz, H-5'), 7.04 (1H, d, *J* 1.6 Hz, H-2'), 7.17-7.19 (1H, m, ArH), 7.31 (1H, d, *J* 7.0 Hz, ArH), 7.36 (2H, t, *J* 7.0 Hz, ArH), 7.41-7.46 (3H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.62 (NCH_3), 56.36 (OCH_3), 56.50 (OCH_3), 61.26 (OCH_3), 62.50 (OCH_3), 71.39 (CH_2), 101.67 (CH, Ar), 111.68 (CH, Ar), 114.95 (CH, Ar), 116.72 (C, Ar), 122.48 (CH, Ar), 127.26 (2 x CH, Ar), 128.36 (CH, Ar), 128.92 (2 x CH, Ar), 136.92 (C, Ar), 137.67 (C, Ar), 147.73 (C, Ar), 147.91 (C, Ar), 147.98 (C, Ar), 151.38 (C, Ar), 153.00 (C, Ar), 153.58 (C=N), 162.72 (C=O). ν_{max} (solid)/(cm^{-1}) 2991 (md0, 1667 9st), 1501 (st), 1132 (st), 1102 (st), 801 (st). MS *m/z* (**API-ES**): found 463 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 463.1920 $(M+H)^+$, calculated for $C_{26}H_{27}N_2O_6$ 463.1869.

2-(3-Hydroxy-4-methoxyphenyl)-6,7,8-trimethoxy-3-methyl-3H-quinazolin-4-one **(127t).** This was obtained as a white solid (0.110 g, 0.29 mmol, 92%) from **127s** (0.149 g, 0.32 g mmol) in a similar manner as described for preparation of **127r**, mp 183-185 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 3.52 (3H, s), 3.93 (3H, s), 3.98 (3H, s), 4.02 (3H, s), 4.07 (3H, s), 6.24 (1H, s), 6.92 (1H, d, *J* 8.5 Hz, H-5'), 7.08 (1H, dd, *J* 2.4, 8.5 Hz, H-6'), 7.12 (1H, d, *J* 2.4 Hz, H-2'), 7.48 (1H, s, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.77 (NCH_3), 56.27 (OCH_3), 56.50 (OCH_3), 61.59 (OCH_3), 62.49 (OCH_3), 101.70 (CH, Ar), 110.72 (CH, Ar), 115.36 (CH, Ar), 116.75 (C, Ar), 121.04 (CH, Ar), 128.84 (C, Ar), 137.66 (C, Ar), 145.87 (C, Ar), 147.77 (C, Ar), 148.00 (C, Ar), 148.32 (C, Ar), 153.03 (C, Ar), 153.78 (C=N), 162.69 (C=O). ν_{max} (solid)/(cm^{-1}) 3243 (st), 1653 (st), 1586 (st), 1512 (st), 1484 (st), 1440 (st), 1371 (st), 1344 (st), 1288 (st), 1242 (st), 1136 (st). MS *m/z* (**API-ES**): found 373 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 373.1402 $(M+H)^+$, calculated for $C_{19}H_{21}N_2O_6$ 373.1400.

4-Benzyloxycarbonylamino-3-(4-chloro-phenyl)-butyric acid (138)[417]

t-Butyl dicarbonate (5.32 g, 24.41 mmol) was added to a stirred solution of Baclofen (**134**) (5.20 g, 24.41 mmol) and NaOH 1M (73 ml) in water (73 ml) and 1,4- dioxane (73 ml) at 0 °C. After stirring for 4 h at room temperature, a 10 % aqueous solution of citric acid was added until pH 3. The formed white solid was filtered, washed with water (50 ml) and dried. Pure **138** was obtained without further purification (6.43 g, 20.56 mmol, 84%), mp 139-141 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 1.37 (9H, s, $C(\underline{CH_3})_3$), 2.50 (1H, dd, *J* 8.8, 15.2 Hz, CH, $CH_2CHC\underline{H_2}CO$), 2.66 (1H, dd, *J* 5.0, 15.4 Hz, CH, $CH_2CHC\underline{H_2}CO$), 3.12-3.27 (3H, m, $C\underline{H_2}C\underline{H}CH_2CO$), 7.22 (2H, d, *J* 8.4 Hz, 2 x Ar-H), 7.27 (2H, d, *J* 8.4 Hz, 2 x Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.84 ($C(\underline{CH_3})_3$), 39.52 ($CH_2CH\underline{C}H_2CO$), 41.99 ($CH_2\underline{C}HCH_2CO$), 45.88 ($C\underline{H_2}CHCH_2CO$), 78.25 ($\underline{C}(CH_3)_3$), 128.70 (2 x CH, Ar), 130.34 (2 x CH, Ar), 131.59 (C, Ar), 142.08 (C, Ar), 156.26 (C=O), 173.80 (C=O). ν_{max} (solid)/(cm^{-1}) 3290 (md), 1699 (st), 1638 (md), 1398 (md).

Benzyl [2-(4-Chloro-phenyl)-3-phenylcarbamoyl-propyl]-carbamate (139a)

2-(7-Aza-1H-benzotriazole-1-Yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate (HATU) (0.315 g, 0.9087 mmol) and diisopropylamine (0.340 g, 2.63 mmol) were added to a solution of **138** (0.206 g, 0.657 mmol) and aniline (0.051 g, 0.548 mmol) in dry DMF (5 ml) at room temperature under Ar. After stirring overnight at room temperature, water (10 mL) was added and the reaction mixture was extracted with ethyl acetate (2 x 15 ml). The organic extracts were collected, dried over Na_2SO_4 and the solvent removed under reduced pressure. Chromatography on silica gel (70:30 hexane/ethyl acetate, R_f 0.30) afforded **139a** as a white solid (0.120 g, 0.307 mol, 47%), mp 165-167 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (9H, s, $C(\underline{CH_3})_3$), 2.55 (1H, dd, *J* 5.0, 13.8 Hz, CH, $CH_2CHC\underline{H_2}CO$), 2.75 (1H, dd, *J* 8.4, 13.6 Hz, CH, $CH_2CHC\underline{H_2}CO$), 3.16-3.39 (2H, m, 2 x CH, $C\underline{H_2}CHCH_2CO$), 3.52-3.57 (1H, m,

$CH_2C\underline{H}CH_2CO$), 4.60 (1H, bs, $N\underline{H}COOC(CH_3)_3$), 7.07-7.13 (3H, m, ArH), 7.29-7.32 (4H, m, ArH), 7.54 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 8.61 (1H, bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) δ 28.54 ($C(\underline{C}H_3)_3$), 41.70 ($CH_2CH\underline{C}H_2CO$), 42.78 ($CH_2\underline{C}HCH_2CO$), 45.12 ($\underline{C}H_2CHCH_2CO$), 80.30 ($\underline{C}(CH_3)_3$), 120.08 (2 x CH, Ar), 124.38 (2 x CH, Ar), 129.10 (2 x CH, Ar), 129.14 (2 x CH, Ar), 129.30 (CH, Ar), 133.10 (C, Ar), 138.36 (C, Ar), 140.49 (C, Ar), 157.22 (C=O), 169.82 (C=O). ν_{max} (solid)/(cm^{-1}) 3323 (st), 1640 (st), 1614 (st), 1220 (st), 1118 (st). MS *m/z* (API-ES): found 389 $(M+H)^+$ (100%), 391 $(M^{37}C+H)^+$ (35%). HRMS *m/z* (API-ES): found 389.1628 $(M+H)^+$, calculated for $C_{21}H_{26}ClN_2O_3$ 389.1632

Ethyl 4-[4-benzyloxycarbonylamino-3-(4-chloro-phenyl)-butyrylamino]-benzoate **(139b).** The compound was prepared from **138** (2.4 g, 7.6 mmol) and ethyl-4-aminobenzoate (1.26 g, 7.66 mmol) in a similar manner as described for preparation of **139a**. Chromatography on silica gel (70:30 hexanes/ethyl acetate, R_f 0.20) gave **139b** as a brown solid (2.54 g, 5.52 mmol, 72%), mp 145-147 °C. ^1H NMR (400 MHz, CD_3OD) δ 1.35 (9H, s, $C(\underline{CH_3})_3$), 1.35 (3H, t, *J* 6.8 Hz, $CH_2C\underline{H_3}$), 2.63 (1H, dd, *J* 8.6, 14.6 Hz, CH, $CH_2CHC\underline{H_2}CO$), 2.77 (1H, dd, *J* 6.2, 14.6 Hz, CH, $CH_2CHC\underline{H_2}CO$), 3.35-3.40 (1H, m, $CH_2C\underline{H}CH_2CO$), 4.31 (2H, q, *J* 7.2 Hz, $C\underline{H_2}CH_3$), 7.14 (2H, d, *J* 8.4 Hz, 2 x Ar-H), 7.24 (2H, d, *J* 8.4 Hz, 2 x Ar-H), 7.28 (2H, d, *J* 8.6 Hz, 2 x Ar-H), 7.57 (2H, d, *J* 8.8 Hz, 2 x Ar-H), 7.91 (2H, d, *J* 8.6 Hz, 2 x Ar-H), 7.91 (2H, d, *J* 8.8 Hz, 2 x Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.56 ($CH_2\underline{C}H_3$), 28.56 ($C(\underline{C}H_3)_3$), 40.92 ($CH_2CH\underline{C}H_2CO$), 42.77 ($CH_2\underline{C}HCH_2CO$), 45.97 ($\underline{C}H_2CHCH_2CO$), 61.04 ($\underline{C}(CH_3)_3$), 80.56 ($\underline{C}H_2CH_3$), 119.07 (2 x CH, Ar), 125.90 (C, Ar), 129.08 (2 x CH, Ar), 129.17 (2 x CH, Ar), 130.90 (2 x CH, Ar), 133.17 (C, Ar), 140.35 (C, Ar), 142.65 (C, Ar), 157.54 (C=O), 166.47 (C=O), 170.32 (C=O). ν_{max} (solid)/(cm^{-1}) 3353 (md), 1685 (st), 1685 (st), 1661 (st), 1524 (st), 1273 (st), 1249 (st), 1188 (st), 769 (md). MS *m/z* (**API-ES**): found 461 $(M^{35}C+H)^+$ (100%), 463 $(M^{37}C+H)^+$ (30%). HRMS *m/z* (**API-ES**): found 461.1841 $(M+H)^+$, calculated for $C_{24}H_{30}ClN_2O_5$ 461.1843

3-(4-Chloro-phenyl)-N-phenyl-4-(toluene-4-sulfonylamino)-butyramide (140a)

A solution of **139a** (0.106 g, 0.271 mmol) in DCM (2 ml) and TFA (2 ml) was stirred for 2 h at room temperature, and the solvent removed under reduced pressure. The crude material was dissolved in 1,4-dioxane:H_2O 1:1 (5 ml) and K_2CO_3 (0.225 g, 1.63 mmol, 6 eq) was added followed by tosyl chloride (0.056 g, 0.298 mmol, 1.1 eq). After stirring for 2 h at room temperature, the removed under reduced pressure. Water (10 ml) was added and the precipitae was separated by filtration and dried in vacuo. Pure **140a** was as a white solid obtained without further purification (0.079 g, 0.179 mmol, 66%), mp 200-202 °C. ^1H NMR (400 MHz, CD_3OD) δ 2.39 (3H, s, $C\underline{H_3}$), 2.56 (1H, dd, *J* 9.2, 14.4 Hz, CH, $CH_2CHC\underline{H_2}CO$), 2.77 (1H, dd, *J* 6.8, 14.4 Hz, CH, $CH_2CHC\underline{H_2}CO$), 3.06 (1H, dd, *J* 8.2, 13.0 Hz, CH, $C\underline{H_2}CHCH_2CO$), 3.15 (1H, dd, *J* 6.8, 13.2 Hz, CH, $C\underline{H_2}CHCH_2CO$) 7.03-7.07 (1H, m, ArH),

7.15 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.21-7.26 (4H, s, ArH), 7.29 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.36-7.38 (2H, m, ArH), 7.62 (2H, d, *J* 8.4 Hz, 2 x CH, Ar). ^{13}C NMR (400 MHz, DMSO-d_6) δ 21.63 (Ar-$\underline{C}H_3$), 40.73 ($\underline{C}H_2CO$), 41.87 ($CH_2\underline{C}HCH_2$), 48.13 ($\underline{C}H_2NH$), 119.79 (2 x CH, Ar), 123.80 (CH, Ar), 127.14 (2 x CH, Ar), 128.80 (2 x CH, Ar), 129.29 (2 x CH, Ar), 130.23 (2 x CH, Ar), 130.36 (2 x CH, Ar), 131.80 (C, Ar), 138.14 (C, Ar), 139.64 (C, Ar), 141.53 (C, Ar), 143.20 (C, Ar), 169.81 (C=O). ν_{max} (solid)/(cm^{-1}) 3340 (md) (N-H), 3141 (md) (N-H), 1665 (st) (C=O), 1598 (st), 1547 (st), 1493 (md), 1444 (st), 1154 (st), 1087 (md). MS *m/z* (**API-ES**): found 443 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 443.1210 $(M+H)^+$, calculated for $C_{23}H_{24}ClN_2O_3S$ 443.1196.

Ethyl 4-[3-(4-chloro-phenyl)-4-(toluene-4-sulfonylamino)-butyrylamino]-benzoate **(140b)**. This was obtained as a white solid (0.125 g, 0.240 mmol, 96%) from **139b** (0.115 g, 0.250 mmol) and tosyl chloride (0.048 g, 0.250 mmol) in a similar manner as described for preparation of **140a**, mp 187-189 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 1.36 (3H, t, *J* 7.2 Hz, $CH_2C\underline{H}_3$), 2.39 (3H, s, $C\underline{H}_3$), 2.60 (1H, dd, *J* 8.8, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.82 (1H, dd, *J* 6.0, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.06 (1H, dd, *J* 8.0, 13.2 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.14 (1H, dd, *J* 6.8, 13.2 Hz, CH, $C\underline{H}_2CHCH_2CO$) 4.32 (2H, q, *J* 7.2 Hz, $C\underline{H}_2CH_3$), 7.15 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.22 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.29 (2H, d, *J* 8.4 Hz), 7.54 (2H, d, *J* 8.8 Hz), 7.62 (2H, d, *J* 8.4 Hz), 7.91 (2H, d, *J* 9.2 Hz). ^{13}C NMR (400 MHz, DMSO-d_6) δ 14.88 ($CH_2\underline{C}H_3$), 21.62 ($\underline{C}H_3$), 40.74 ($CH_2CH\underline{C}H_2CO$), 41.76 ($CH_2\underline{C}HCH_2CO$), 48.25 ($\underline{C}H_2CHCH_2CO$), 61.08 ($\underline{C}H_2CH_3$), 119.06 (2 x CH, Ar), 124.78 (C, Ar), 127.34 (2 x CH, Ar), 128.83 (2 x CH, Ar), 130.23 (2 x CH, Ar), 130.36 (2 x CH, Ar), 130.84 (2 x CH, Ar), 131.83 (C, Ar), 138.15 (C, Ar), 141.42 (C, Ar), 143.20 (C, Ar), 143.95 (C, Ar), 165.98 (C=O), 170.47 (C=O). ν_{max} (solid)/(cm^{-1}) 3320 (md), 3292 (md), 1712 (st) (C=O), 1677 (st) (C=O), 1598 (md), 1540 (st), 1315 (st), 1272 (st), 1149 (st), 1104 (st). MS *m/z* (API-ES): found 515 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 515.1411 $(M+H)^+$, calculated for $C_{26}H_{28}ClN_2O_5S$ 515.1407.

Ethyl 4-[3-(4-chloro-phenyl)-4-(4-phenoxybenzenesulfonylamino)butyrylamino]benzoate **(140c)**. This was obtained as a white solid (0.119 g, 0.201 mmol, 65%) from **140b** (0.141 g, 0.307 mmol) and 4-phenoxybenzenesulfonyl chloride (0.082 g, 0.307 mmol) in a similar manner as described for preparation of **140a**, mp 154-156 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 1.36 (3H, t, *J* 6.8 Hz, $CH_2C\underline{H}_3$), 2.60 (1H, dd, *J* 8.0, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.82 (1H, dd, *J* 6.0, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.09 (1H, dd, *J* 7.8, 13.4 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.16 (1H, dd, *J* 6.4, 13.2 Hz, CH, $C\underline{H}_2CHCH_2CO$), 4.32 (2H, q, *J* 7.2 Hz, $C\underline{H}_2CH_3$), 6.99 (2H, d, *J* 8.0 Hz, 2 x CH, Ar), 7.06-7.08 (1H, m, ArH), 7.18 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.18 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.19-7.24 (2H, m), 7.56 (2H, d, *J* 8.8 Hz), 7.72 (2H, d, *J* 8.0 Hz), 7.91 (2H, d, *J* 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) d 14.55 ($CH_2\underline{C}H_3$), 40.49 ($CH_2CH\underline{C}H_2CO$), 41.48 (CH_2CHCH_2CO), 46.69 ($\underline{C}H_2CHCH_2CO$), 61.10 ($\underline{C}H_2CH_3$),

117.91 (2 x CH, Ar), 119.70 (2 x CH, Ar), 120.56 (2 x CH, Ar), 125.31 (CH, Ar), 129.12 (2 x CH, Ar), 129.32 (2 x CH, Ar), 129.39 (2 x CH, Ar), 130.43 (2 x CH, Ar), 130.98 (2 x CH, Ar), 133.12 (C, Ar), 133.61 (C, Ar), 139.28, (C, Ar), 141.97 (C, Ar), 155.15 (C, Ar), 162.06 (C, Ar), 162.06, (C, Ar)., 166.31 (C=O), 169.90 (C=O). ν_{max} (solid)/(cm^{-1}) . MS *m/z* (**API-ES**): found 593 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 593.1513 $(M+H)^+$, calculated for $C_{31}H_{30}ClN_2O_6S$ 593.1513.

4-[3-(4-Chlorophenyl)-4-(toluene-4-sulfonylamino)butyrylamino]benzoic acid (141a)
A solution of **140b** (0.023 g, 0.044 mmol) in methanol (1 ml) and THF (1 ml) was stirred in presence of NaOH 1M (1 ml) overnight at room temeprature. The solution remaining was concentrated in vacuo. HCl 1M (1 ml) was added and the formed white solid was filtered, washed with water (5 ml) and dried. The pure compound **141a** was obtained without further purification (0.015 g, 0.030 mmol, 68 %), mp 250-252 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 2.39 (3H, s, $C\underline{H}_3$), 2.60 (1H, dd, *J* 8.8, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.82 (1H, dd, *J* 6.4, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.06 (1H, dd, *J* 8.0, 13.2 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.14 (1H, dd, *J* 6.8, 13.2 Hz, CH, $C\underline{H}_2CHCH_2CO$), 7.16 (2H, d, *J* 8.8 Hz), 7.23 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.29 (2H, d, *J* 8.2 Hz, 2 x CH, Ar), 7.54 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 7.62 (2H, d, *J* 8.2 Hz), 7.92 (2H, d, *J* 9.0 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.62 (CH_3), 40.81 ($CH_2CH\underline{C}H_2CO$), 41.76 ($CH_2\underline{C}HCH_2CO$), 48.08 ($\underline{C}H_2CHCH_2CO$), 118.95 (2 x CH, Ar), 125.69 (C, Ar), 127.13 (2 x CH, Ar), 128.83 (2 x CH, Ar), 130.24 (2 x CH, Ar), 130.36 (C, Ar), 131.00 (2 x CH, Ar), 138.10 (C, Ar), 141.42 (C, Ar), 143.22 (C, Ar), 143.64 (C, Ar), 167.56 (C=O), 170.39 (C=O). ν_{max} (solid)/(cm^{-1}) 1864 (st), 1665 (st), 1653 (st), 1604 (st), 1519 (st), 1455 (st), 1324 (st), 1290 (st), 1164 (st), 1151 (st). MS *m/z* (**API-ES**): found 485 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 485.0935 $(M-H)^-$, calculated for $C_{24}H_{22}ClN_2O_5S$ 485.0938.

4-[3-(4-chlorophenyl)-4-(4-phenoxybenzenesulfonylamino)butyrylamino]-benzoic acid **(141b)**. A solution of **140c** (0.065 g, 0.107 mmol) in methanol (1.5 ml) and THF (1.5 ml) was stirred in presence of NaOH 1M (1 ml) for 2 h at 80 °C. After cooling to room temperature, the solution remaining was concentrated in vacuo. HCl 1M (1.5 ml) was added and the formed white solid was filtered, washed with water (10 ml) and dried. The pure compound **141b** was obtained without further need for purification (0.050 g, 0.088 mmol, 83 %), mp 119-121 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 2.61 (1H, dd, *J* 8.4, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.82 (1H, dd, *J* 6.0, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.09 (1H, dd, *J* 7.8, 13.4 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.16 (1H, dd, *J* 6.4, 13.2 Hz, CH, $C\underline{H}_2CHCH_2CO$), 6.99 (2H, d, *J* 9.0 Hz, 2 x CH, Ar), 7.06-7.08 (2H, m, ArH), 7.17-7.24 (5H, m, ArH), 7.47.44 (2H, m, ArH), 7.54 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 7.72 (2H, d, *J* 9.0 Hz, 2 x CH, Ar), 7.91 (2H, d, *J* 9.2 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, CD_3OD) δ 40.59 ($CH_2\underline{C}HCH_2CO$), 42.01 ($CH_2CH\underline{C}H_2CO$), 47.61 ($\underline{C}H_2CHCH_2CO$), 117.41 (2 x CH, Ar), 118.97 (C, Ar), 120.13 (2

x CH, Ar), 124.80 (CH, Ar), 125.76 (C, Ar), 128.43 (2 x CH, Ar), 129.09 (2 x CH, Ar), 129.36 (2 x CH, Ar), 130.12 (2 x CH, Ar), 130.53 (C, Ar), 132.57 (C, Ar), 134.32 (C, Ar), 140.20 , 142.87 (C, Ar), 155.55 (C, Ar), 161.61 (C, Ar), 168.22 (C=O), 171.80 (C=O). ν_{max} (solid)/(cm^{-1}) 3359 (st), 1686 (st), 1595 (st), 1530 (st), 1487 (st), 1410 (md), 1318 (md), 1246 (st), 1150 (st), 1092 (md). MS *m/z* (**API-ES**): found 563 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 563.1043 $(M-H)^-$, calculated for $C_{29}H_{24}ClN_2O_6S$ 563.1044.

(*E*)-3-(2-Naphthyl)propenoic acid (146b)[379]

Malonic acid (7.6 g, 72.7 mmol, 2.2 eq) was added to a solution of 2-naphthaldehyde (5.1 g, 32.7 mmol) in pyridine (40 ml) and the mixture was stirred at 100 °C overnight. After cooling to room temperature, the reaction mixture was poured into 2N HCl (200 ml). The white precipitate was filtered, washed with water (50 ml) and dried. Pure acid was obtained after recrystallization from ethanol as a white solid (4.0 g, 20.2 mmol, 62%), mp 204-206 °C (lit[379] 207-208 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.64 (1H, d, *J* 16.0 Hz, CH), 7.53-7.55 (2H, m, ArH), 7.73 (1H, d, *J* 16.0 Hz, CH), 7.84-7.93 (4H, m, 4 x CH, ArH), 8.16 (1H, s, CH, ArH) 12.43 (1H, bs, OH).

***Methyl (E)-4-(2-Carboxy-vinyl)-benzoate* (146a).**[379] This was obtained as a yellow solid (0.621 g, 3.11 mmol, 34%) from benzaldehyde (1.562 g, 9.51 mmol) and malonic acid (2.177g, 20.92 mmol, 2.2 equiv) in a similar manner as described for preparation of **146b**, mp 259-261 °C (lit[418] 246-247 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (3H, s, OCH_3), 6.64 (1H, d, *J* 16.0 Hz, CH), 7.62 (1H, d, *J* 16.0 Hz, CH), 7.82 (1H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.94 (1H, d, *J* 8.4 Hz, 2 x CH, Ar), 12.60 (1H, s, OH).

(*E*)-3-(2-Naphthyl)propenoyl chloride (147b)[375]

Thionyl chloride (10 ml) was added to a suspension of **146a** (3.4 g, 18.6 mmol) in anhydrous toluene (40 ml) at room temperature under Ar. The reaction mixture was stirred at 100 °C for 2 h. The solvent was removed under reduced pressure to provide a white solid (3.9 g, 18.05 mmol, 97%). The acid chloride was used in the next step without further purification.

***Methyl (E)-4-(2-chlorocarbonyl-vinyl)benzoate* (147a).** This was obtained as a yellow solid (0.589 g, 2.629 mmol, 89%) from corresponding acid **146a** (0.612 g, 2.956 mmol) in a similar manner as described for preparation of **147b**. The acid chloride was used in the next step without further purification.

***4-Nitrocinnamoyl chloride* (147c).** This was obtained as a yellow solid (4.2 g, 19.90 mmol, 98%) from corresponding acid **146c** (3.9 g, 20.19 mmol) in a similar manner as described for preparation of **147b**. The acid chloride was used in the next step without further purification.

***4-Chlorocinnamoyl chloride* (147d).** This was obtained as an off-white solid (1.94 g, 10.20 mmol, 99%) from corresponding acid **146d** (1.76 g, 10.22 mmol) in a similar manner as described for preparation of **147b**. The acid chloride was used in the next step without further purification.

***4-Methoxycinnamoyl chloride* (147e).** This was obtained as a yellow solid (3.2 g, 16.32 mmol, 94%) from corresponding acid **146e** (3.1 g, 17.39 mmol) in a similar manner as described for preparation of **147b**. The acid chloride was used in the next step without further purification.

***3,4,5-Trimethoxycinnamoyl chloride* (147f).** This was obtained as a yellow solid (1.753 g, 6.84 mmol, 92%) from corresponding acid **146f** (1.762 g, 7.40 mmol) in a similar manner as described for preparation of **147b**. The acid chloride was used in the next step without further purification.

Methyl 4-amino-2-hydroxybenzoate (148c)[376,377]

A solution of 4-amino-2-hydroxybenzoic acid (4-aminosalicylic acid) (2.5 g, 16.3 mmol) and concentrated sulfuric acid (3.5 ml) in methanol (50 ml) was heated under reflux overnight. After addition of saturated sodium bicarbonate solution (until the evolution of CO_2 ceased) the reaction mixture was filtered. The filtrate was washed with water (50 ml), dried under vacuum to afford methyl 4-amino-2-hydroxybenzoate (**148c**) as a pale brown solid (1.72 g, 10.3 mmol, 63%), mp 110-112 °C (lit[419] 197 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.76 (3H, s, OCH_3), 5.96 (1H, d, *J* 2.3 Hz, CH-3), 6.09 (1H, dd, *J* 2.3, 8.9, Hz, CH-5), 6.12 (2H, bs, NH_2), 7.42 (1H, d, *J* 8.9 Hz, CH-6), 10.74 (1H, s, OH).

Methyl 4-aminobenzoate (148e)[378]

Thionyl chloride (6 ml, 1.5 eq) was added dropwise over 20 min to a stirred solution of 4-aminobenzoic acid (5.7 g, 41.6 mmol) in methanol (200 ml) under ice-cooling. The mixture was stirred at room temperature overnight. The methanol was removed under reduce pressure. The resultant residue was diluted with ethyl acetate (200 ml) and then a saturated sodium bicarbonate solution (200 ml) was added to the solution. The aqueous phase was separated and extracted with ethyl acetate (200 ml). The organic extracts were collected, dried over Na_2SO_4, and the solvent removed under reduced pressure to afford the ester **148e** in pure form (4.7 g, 31.1 mmol, 77%) as an off-white solid, mp 105-107 °C (lit[420] 107-110 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (3H, s, OCH_3), 4.05 (2H, s, NH_2), 6.64 (2H, d, *J* 9.0 Hz, 2 x CH, Ar), 7.85 (2H, d, *J* 9.0 Hz, 2 x CH, Ar).

***Methyl 3-aminobenzoate* (148d).**[378] This was obtained as brown oil (4.4 g, 29.13 mmol, 75%) from the corresponding 3-aminobenzoic acid (5.3 g, 38.68 mmol) in a similar manner as described for preparation of **148e**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (2H, bs, NH_2), 3.89 (3H, s, OCH_3), 6.85 (1H, ddd, *J* 1.0, 2.4, 7.7 Hz, ArH), 7.21 (1H, t, *J* 7.7 Hz, ArH), 7.35 (1H, m, ArH), 7.41-7.43 (1H, m, ArH).

Methyl 2-[3-(4-chlorophenyl)acryloylamino]benzoate (145g)

Anhydrous pyridine (0.227 g, 2.88 mmol, 1.2 eq) and methyl anthranilate **148a** (0.365 g, 2.40 mmol) were added to a solution of 4-chlorocinnamoyl chloride (**147d**) (0.458 g, 2.40 mmol) in anhydrous DCM (15 ml) under Ar. The reaction mixture was stirred at room temperature overnight and the mixture was poured in 2N HCl (20 ml). The product was extracted with DCM (2 x 20 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure. The pure compound **145g** was obtained after trituration with cold methanol (10 ml) as a white solid (0.617 g, 2.02 mmol, 84%), mp 122-124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96 (3H, s, OCH_3), 6.59 (1H, d, *J* 15.6 Hz, CH), 7.09-7.14 (1H, m, ArH), 7.37 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.52 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.53-7.61 (1H, m, ArH), 7.70 (1H, d, *J* 15.6 Hz, CH), 8.0 (1H, dd, *J* 1.2, 8.0 Hz, ArH), 8.86 (1H, dd, *J* 1.2, 8.0 Hz, ArH), 11.40 (1H, bs, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.64 (OCH_3), 115.14 (C, Ar), 120.81 (CH, Ar), 122.76 (CH), 122.90 (CH, Ar), 129.35 (2 x CH, Ar), 129.45 (2 x CH, Ar), 131.12 (CH, Ar), 133.40 (C, Ar), 135.03 (CH, Ar), 136.06 (C, Ar), 141.07 (CH), 141.97 (C, Ar), 164.39 (C, Ar), 169.19 (C=O). ν_{max} (solid)/(cm^{-1}) 3302 (md), 1697 (st), 1683 (st), 1628 (st), 1604 (md), 1589 (st), 1528 (st), 1433 (st), 1254 (st), 1155 (md), 1086 (md). MS *m/z* (**API-ES**): found 316 $(M^{35}Cl+H)^+$ (100%), 318 $(M^{37}Cl+H)^+$ (35%). HRMS *m/z* (**API-ES**): found 316.0734 $(M+H)^+$ (100%), calculated for $C_{17}H_{15}ClNO_3$ 316.0740.

***Methyl 4-[2-(2-carboxylphenylcarbamoyl)vinyl]benzoate* (145a).** This was obtained as a white solid (0.306 g, 0.902 mol, 84%) from corresponding acid chloride **147a** (0.240 g, 1.071 mmol) and aniline **148a** (0.178 g, 1.187 mmol) in a similar manner as described for preparation of **145g**, mp 146-148 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 6.70 (1H, d, *J* 15.6 Hz, CH), 7.10-714 (1H, m, ArH), 7.57-7.62 (1H, m, ArH), 7.64 (2H, d, *J* 8.4 Hz, ArH), 7.77 (1H, d, *J* 15.6 Hz, CH), 8.05-8.08 (3H, m, ArH), 8.86 (1H, dd, *J* 1.0, 8.5Hz, ArH), 11.45 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.49 (OCH_3), 52.68 (OCH_3), 115.19 (C, Ar), 120.82 (CH, Ar), 123.02 (CH), 124.55 (CH, Ar), 128.13 (CH, Ar), 130.31v (CH, Ar), 131.14 (CH, Ar), 131.10 (C, Ar), 135.05 (CH, Ar), 139.16 (C, Ar), 141.09 (CH), 141.89 (C, Ar), 164.11 (C=O), 166,75 (C=O), 169.19 (C=O). ν_{max} (solid)/(cm^{-1}) 3266 (st), 1710 (st), 1677 (st), 1620 (st), 1605 (st), 1590 (st), 1527 (st), 1446 (st), 1434 (st), 1256 (st). MS *m/z* (**API-ES**): found 340 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 340.1183 $(M+H)^+$, calculated for $C_{19}H_{18}NO_5$ 340.1185.

Ethyl 4-(3-naphthalen-2-yl-acryloylamino)benzoate **(145b).** This was obtained from the corresponding acid chloride **147b** (0.757 g, 3.50 mmol) and aniline **148g** (0.577 g, 3.50 mmol) in a similar manner as described for preparation of **145g**. After stirring overnight at room temperature, the white precipitate was filtered, washed with DCM (10 ml) and dried vacuum to give the amide **145b** (0.960 g, 2.78 mmol, 80%) as a white solid, mp 173-175 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (3H, t, *J* 7.2 Hz, $CH_2\underline{CH_3}$), 4.28 (2H, t, *J* 7.2 Hz, $\underline{CH_2}CH_3$), 6.97 (1H, d, *J* 15.4 Hz, CH), 7.54-7.57 (2H, m, ArH), 7.7 (1H, d, *J* 15.4 Hz, CH), 7.77 (1H, dd, *J* 1.6, 9.2 Hz, CH, Ar), 7.84 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.93-7.97 (5H, m, ArH), 8.15 (1H, s, ArH), 10.58 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.88 ($CH_2\underline{C}H_3$), 61.09 , ($\underline{C}H_2CH_3$), 119.30 (2 x CH, Ar), 122.91 (CH), 124.18 (CH, Ar), 124.99 (C, Ar), 127.47 (CH, Ar), 127.83 (CH, Ar), 128.38 (CH, Ar), 129.09 (CH, Ar), 129.36 (CH, Ar), 130.05 (CH, Ar), 130.98 (CH, Ar), 132.82 (C, Ar), 133.68 (C, Ar), 134.26 (C, Ar), 141.64 (CH), 144.33 (C, Ar), 164.69 (C=O), 166.01 (C=O). ν_{max} (solid)/(cm^{-1}) 3357 (st), 1702 (st), 1660 (st), 1619 (st), 1607 (st), 1591 (st), 1521 (st), 1403 (st), 1282 (st). MS *m/z* (**API-ES**): found 346 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 346.1443 $(M+H)^+$ (100%), calculated for $C_{22}H_{20}NO_3$ 346.1440.

Methyl 2-hydroxy-4-(3-naphthalen-2-yl-acryloylamino)benzoate **(145c).** This was obtained from corresponding acid chloride **147b** (0.320 g, 1.48 mmol) and aniline **148b** (0.247 g, 1.48 mmol) in a similar manner as described for preparation of **145g**. After stirring overnight at room temperature, the white solid was filtered, washed with DCM (10 ml) and dried under vacuum to give the amide **145c** (0.400 g, 1.15 mmol, 79%) as an off-white solid, mp 210-212 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.86 (3H, s, OCH_3), 6.31 (1H, d, *J* 15.6 Hz, CH), 7.17 (1H, dd, *J* 1.8, 8.8 Hz, H-6'), 7.53 (1H, d, *J* 1.8 Hz, H-2'), 7.54-7.57 (2H, m, ArH), 7.74-7.79 (3H, m, ArH), 7.92-7.99 (3H, m, Ar), 8.15 (1H, s, ArH), 10.54 (1H, s, NH), 10.65 (1H, s, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.01 (OCH_3), 106.95 (CH, Ar), 108.27 (C, Ar), 111.30 (CH, Ar), 122.78 (CH), 124.21 (CH, Ar), 127.54 (CH, Ar), 127.92 (CH, Ar), 128.40 (CH, Ar), 129.12 (CH, Ar), 129.39 (CH, Ar), 130.12 (CH, Ar), 131.56 (CH, Ar), 132.76 (C, Ar), 133.67 (C, Ar), 134.30 (C, Ar), 141.91 (CH), 146.35 (C, Ar), 161.91 (C, Ar), 164.84 (C=O), 169.70 (C=O). ν_{max} (solid)/(cm^{-1}) 3352 (st), 1692 (st), 1662 (st), 1622 (st), 1597 (st), 1507 (st), 1445 (st), 1362 (st), 1265 (st), 1188(st), 1143 (st), 1095 (st). MS *m/z* (**API-ES**): found 346 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 346.1088 $(M-H)^-$ (100%), calculated for $C_{21}H_{16}NO_4$ 346.1079.

Methyl 2-[3-(4-nitrophenyl)acryloylamino]benzoate **(145d).** This was obtained from corresponding acid chloride **147c** (0429 g, 2.033 mmol) and aniline **148a** (0.306 g, 2.033 mmol) in a similar manner as described for preparation of **145g**. After stirring overnight at room temperature, the yellow precipitate was filtered, washed with DCM (10 ml) and dried under vacuum to give the amide **145d** (0.479 g, 1.47 mmol, 72%) as a yellow solid, mp 223-

225 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.86 (3H, s, OCH_3), 7.15 (1H, d, *J* 15.6 Hz, CH), 7.21-7.25 (1H, m, ArH), 7.61-7.64 (1H, m, ArH), 7.71 (1H, d, *J* 15.6 Hz, CH), 7.94 (1H, dd, *J* 1.6, 7.2 Hz, ArH), 8.0 (2H, d, *J* 8.0 Hz, 2 x CH, Ar), 8.25 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 8.35 (1H, d, *J* 8.0 Hz, ArH), 10.90 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 53.17 (OCH_3), 118.93 (CH, Ar), 122.29 (C, Ar), 124.43 (CH, Ar), 124.73 (2 x CH, Ar), 127.19 (CH), 129.91 (2 x CH, Ar), 131.27 (CH, Ar), 134.63 (CH, Ar), 139.55 (CH), 140.01 (C, Ar), 141.71 (C, Ar), 148.49 (C, Ar), 163.85 (C=O), 168.16 (C=O). ν_{max} (solid)/(cm^{-1}) 3264 (md), 1689 (st), 1675 (st), 1588 (st), 1500 (st), 1444 (md), 1314 (st), 1235 (st). MS *m/z* (**API-ES**): found 327 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 327.0978 $(M+H)^+$ (100 %), calculated for $C_{17}H_{15}N_2O_5$ 327.0981.

Methyl 3-[3-(4-nitrophenyl)acryloylamino]benzoate **(145e).** This was obtained from corresponding acid chloride **147c** (0.660 g, 3.12 mmol) and aniline **148d** (0.471 g, 3.12 mmol) in a similar manner as described for preparation of **145g**. After stirring overnight at room temperature, the yellow precipitate was filtered, washed with DCM (10 ml) and dried under vacuum to give the amide **1435e** (0.810 g, 2.48 mmol, 80%) as a yellow solid, mp 224-225 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (3H, s, OCH_3), 6.98 (1H, d, *J* 16.0 Hz, CH), 7.49 (1H, t, *J* 7.9 Hz, ArH), 7.66 (1H, d, *J* 7.9 Hz, CH, Ar), 7.70 (1H, d, *J* 16.0 Hz, CH), 7.88 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.94 (1H, dd, *J* 1.0, 7.9 Hz, ArH), 8.28 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 8.35 (1H, s, ArH), 10.65 (1H, bs, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.90 (OCH_3), 120.45 (CH, Ar), 124.38 (CH, Ar), 124.86 (4 x CH, Ar), 126.88 (CH), 129.50 (CH, Ar), 130.04 (CH, Ar), 130.87 (CH, Ar), 138.79 (CH), 140.08 (C, Ar), 141.83 (C, Ar), 148.38 (C, Ar), 163.75 (C=O), 166.72 (C=O). ν_{max} (solid)/(cm^{-1}) 3370 (st), 1713 (st), 1686 (st), 1591 (st), 1544 (st), 1428 (st), 1337 (st), 1227 (st). MS *m/z* (**API-ES**): found 327 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 327.0984 $(M+H)^+$ (100%), calculated for $C_{17}H_{15}N_2O_5$ 327.0981.

Methyl 4-[3-(4-nitrophenyl)acryloylamino]benzoate **(145f).** This was obtained from corresponding acid chloride **147c** (0.325 g, 1.54 mmol) and aniline **148e** (0.232 g, 1.54 mmol) in a similar manner as described for preparation of **145g**. After stirring overnight at room temperature, the yellow precipitate was filtered, washed with DCM (10 ml) and dried under vacuum to give the amide **145f** (0.421 g, 1.29 mmol, 84%) as a yellow solid, mp 252-254 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (3H, s, OCH_3), 7.01 (1H, d, *J* 15.6 Hz, CH), 7.72 (1H, d, *J* 15.6 Hz, CH), 7.82 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 7.89 (2H, d, *J* 8.7 Hz, 2 x CH, Ar), 7.96 (2H, d, *J* 8.7 Hz, 2 x CH, Ar), 8.28 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 10.69 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.40 (OCH_3), 119.46 (2 x CH, Ar), 124.88 (4 x CH, Ar), 125.00 (C, Ar), 126.78 (CH), 129.58 (2 x CH, Ar), 131.07 (2 x CH, Ar), 139.20 (CH), 141.76 (C, Ar), 144.05 (C, Ar), 148.46 (C, Ar), 163.98 (C=O), 166.47 (C=O). ν_{max}

(solid)/(cm^{-1}) 3319 (st), 1694 (md), 1678 (st), 1590 (st), 1509 (st), 1403 (st), 1371 (st), 1340 (st), 1319 (st), 1283 (st), 1161 (st) , 1112 (st). MS *m/z* (**API-ES**): found 327 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 327.0977 $(M+H)^+$, calculated for $C_{17}H_{15}N_2O_5$ 327.0981.

***Methyl 3-[3-(4-chlorophenyl)acryloylamino]benzoate* (145h).** This was obtained as a white solid (0.489g, 1.98 mml, 40%) from corresponding acid chloride **147d** (0.688 g, 3.82 mmol) and aniline **148d** (0.576 g, 3.82 mmol) in a similar manner as described for preparation of **145g**, mp 156-158 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91 (3H, s, OCH_3), 6.53 (1H, d, *J* 15.6 Hz, CH), 7.36 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.44 (1H, t, *J* 7.8 Hz, ArH), 7.46 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.57 (1H, s, NH), 7.72 (1H, d, *J* 15.6 Hz, CH), 7.81 (1H, d, *J* 7.8 Hz, ArH), 8.02 (1H, bd, *J* 7.8 Hz, ArH), 8.12 (1H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.51 (OCH_3), 121.22 (CH), 121.43 (CH, Ar), 124.88 (CH, Ar), 125.71 (CH, Ar), 129.31 (4 x CH, Ar), 129.47 (CH, Ar), 131.08 (C, Ar), 133.15 (C, Ar), 136.14 (C, Ar), 138.60 (C, Ar), 141.51 (CH), 164.52 (C=O), 167.02 (C=O). ν_{max} (solid)/(cm^{-1}) 3279 (st), 1715 (st) , 1659 (st), 1626 (st), 1555 (st), 1488 (st), 1281 (st). MS *m/z* (**API-ES**): found 316 $(M^{35}Cl+H)^+$ (100%), 318 $(M^{37}Cl+H)^+$ (70%). HRMS *m/z* (**API-ES**): found 316.0738 $[M+H]^+$ (100%), calculated for $C_{17}H_{15}ClNO_3$ 316.0740.

***Methyl 4-[3-(4-chlorophenyl)acryloylamino]benzoate* (145i).** This was obtained as a white solid (0.230 g, 0.75 mmol, 41%) from corresponding acid chloride **147d** (0.354 g, 1.86 mmol) and aniline **148e** (0.281 g, 1.86 mmol) in a similar manner as described for preparation of **145g**, mp 193-195 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91 (3H, s, OCH_3), 6.52 (1H, d, *J* 16.2 Hz, CH), 7.37 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.47 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.49 (1H, s, NH), 7.70 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.73 (1H, d, *J* 16.2 Hz, CH), 8.04 (2H, d, *J* 8.8 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.29 (OCH_3), 119.25 (2 x CH, Ar), 121.00 (CH), 126.07 (C, Ar), 129.42 (2 x CH, Ar), 129.46 (2 x CH, Ar), 131.14 (2 x CH, Ar), 133.07 (C, Ar), 136.41 (C, Ar), 142.20 (CH Ar), 142.30 (C, Ar), 163.94 (C=O), 166.78 (C=O). ν_{max} (solid)/(cm^{-1}) 3269 , 1716 (st), 1654 (st), 1621 (st), 1590 (md), 1522 (st), 1489 (md), 1434 (md), 1403 (md), 1331 (md), 1273 (st). MS *m/z* (**API-ES**): found 316 $(M^{35}Cl+H)^+$ (100%), 318 $(M^{37}Cl+H)^+$ (35%). HRMS *m/z* (**API-ES**): found 316.0740 $(M+H)^+$ (100%), calculated for $C_{17}H_{15}ClNO_3$ 316.0740.

***Methyl 2-[3-(4-methoxyphenyl)acryloylamino]benzoate* (145j).** This was obtained as a off-white solid (0.900 g, 2.89 mmol, 86%) from corresponding acid chloride **147e** (0.655 g, 3.34 mmol) and aniline **148a** (0.505 g, 3.34 mmol) in a similar manner as described for preparation of **145g**, mp 99-101 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), (1H, s, *J* 15.8 Hz, CH), 6.92 (2H, d, *J* 9.0 Hz, 2 x CH, Ar), 7.07-7.11 (1H, m, ArH), 7.48 (2H, d, *J* 9.0 Hz, 2 x CH, Ar), 7.55-760 (1H, m, ArH), 7.71 (1H, d, *J* 15.8 Hz,

CH), 8.05 (1H, dd, *J* 1.3, 8.5 Hz, ArH), 8.87 (1H, dd, *J* 1.3, 8.5 Hz, ArH), 11.31 (1H, bs, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.55 (OCH_3), 55.55 (OCH_3), 114.50 (2 x CH, Ar), 114.97 (C, Ar), 119.73 (CH), 120.71 (CH, Ar), 122.55 (CH, Ar), 127.60 (C, Ar), 129.89 (2 x CH, Ar), 131.07 (CH, Ar), 134.90 (CH, Ar), 142.09 (CH), 142.24 (C, Ar), 161.38 (C, Ar), 165.02 (C=O), 169.10 (C=O). ν_{max} (solid)/(cm^{-1}) 3255 (md), 1695 (st), 1673 (st), 1626 (st), 1600 (st), 1584 (st), 1509 (st), 1434 (st), 1251 (st). MS *m/z* (API-ES): found 312 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 312.1234 $(M+H)^+$ (100%), calculated for $C_{18}H_{18}NO_4$ 312.1236.

***Methyl 3-[3-(4-methoxyphenyl)acryloylamino]benzoate* (145k).** This was obtained as a white solid (0.600 g, 1.92 mmol, 60%) from corresponding acid chloride **147e** (0.637 g, 3.25 mmol) and aniline **148d** (0.490 g, 3.25 mmol) in a similar manner as described for preparation of **145g**, mp 145-146 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.84 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 6.43 (1H, d, *J* 15.2 Hz, CH), 6.90 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.42 (1H, t, *J* 7.3 Hz, ArH), 7.48 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.55 (1H, bs, NH), 7.73 (1H, d, *J* 15.2 Hz, CH), 7.79 (1H, d, *J* 7.3 Hz, ArH), 8.03 (1H, bd, *J* 7.3 Hz, ArH) 8.12 (1H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.46 (OCH_3), 55.61 (OCH_3), 114.56 (2 x CH, Ar), 118.15 (CH), 120.97 (CH, Ar), 124.68 (CH, Ar), 125.50 (CH, Ar), 127.42 (C, Ar), 129.45 (CH, Ar), 129.86 (2 x CH, Ar), 131.12 (C, Ar), 138.66 (C, Ar), 142.77 (CH), 161.48 (C, Ar), 164.77 (C=O), 166.97 (C=O). ν_{max} (solid)/(cm^{-1}) 3280 (md), 1719 (st), 1657 (st), 1599 (st), 1542 (st), 1253 (st), 1281 (st), 1172 (st). MS *m/z* (**API-ES**): found 312 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 312.1228 $(M+H)^+$ (100%), calculated for $C_{18}H_{18}NO_4$ 312.1236.

***Methyl 4-[3-(4-methoxyphenyl)acryloylamino]benzoate* (145l).** This was obtained as a white solid (0.403 g, 1.29 mmol, 72%) from corresponding acid chloride **147e** (0.355 g, 1.81 mmol) and aniline **148e** (0.273 g, 1.81 mmol) in a similar manner as described for preparation of **145g** mp 179-181 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.84 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.42 (1H, d, *J* 14.8 Hz, CH), 6.90 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.48 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.55 (1H, bs, NH), 7.71 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.74 (1H, d, *J* 14.8 Hz, CH), 8.03 (2H, d, *J* 8.6 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.24 (OCH_3), 55.62 (OCH_3), 114.62 (2 x CH, Ar), 117.96 (CH), 119.14 (2 x CH, Ar), 125.76 (C, Ar), 127.31 (C, Ar), 129.94 (2 x CH, Ar), 131.12 (2 x CH, Ar), 142.62 (C, Ar), 143.25 (CH), 161.62 (C, Ar), 164.64 (C=O), 166.86 (C=O). ν_{max} (solid)/(cm^{-1}) 3160 (md), 1706 (st), 1653 (st), 1602 (st), 1590 (st), 1506 (st), 1403 (st), 1276 (st). MS *m/z* (**API-ES**): found 312 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 312.1239 $(M+H)^+$ (100%), calculated for $C_{18}H_{18}NO_4$ 312.1236.

***Methyl 2-[3-(3,4,5-trimethoxyphenyl)acryloylamino]benzoate* (145m).** This was obtained as a yellow solid (0.462 g, 1.24 mmol, 96%) from corresponding acid chloride **145f** (0.332, 1.29

mmol) and aniline **148a** (0.215, 1.42 mmol) in a similar manner as described for preparation of **145g**, mp 149-150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.82 (3H, s, OCH_3), 3.86 (6H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.46 (1H, d, *J* 15.6 Hz, CH), 7.02-7.07 (1H, m, ArH), 7.50-7.55 (1H, m, ArH), 7.61 (1H, d, *J* 15.6 Hz, CH), 8.00 (1H, dd, *J* 1.2, 8.2 Hz, ArH), 8.81 (1H, dd, *J* 1.2, 8.2 Hz), 11.26 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 51.37 (OCH_3), 55.22 (2 x OCH_3), 59.95 (OCH_3), 104.23 (2 x CH, Ar), 113.80 (C, Ar), 119.62 (CH, Ar), 120.02 (CH, Ar), 121.53 (CH), 129.16 (C, Ar), 129.87 (CH, Ar), 133.78 (CH, Ar), 138.85 (C, Ar), 140.85 (C,, Ar), 141.40 (CH), 152.40 (C, Ar), 163.42 (C=O), 168.02 (C=O). ν_{max} (solid)/(cm^{-1}) 3260 (st), 1681 (st), 1582 (st), 1531 (st), 1505 (st), 1450 (st), 1427 (st), 1414 (st), 1236 (st), 1150 (st). MS *m/z* (**API-ES**): found 372 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 221.0803 $(M-C_8H_8NO_2)^+$, calculated for $C_{12}H_{13}O_4$ 221.0814; found 743.2811 $(2M+H)^+$, calculated for $C_{40}H_{43}N_2O_{12}$ 743.2816; found 372.1430 $(M+H)^+$, calculated for $C_{20}H_{22}NO_6$ 372.1447; found 765.2621 $(2M+Na)^+$, calculated for $C_{40}H_{42}N_2O_{12}Na$ 765.2635.

Methyl 2-(3-phenylacryloylamino)benzoate **(145n).** This was obtained as a white solid (0.700 g, 2.49 mmol, 84%) from corresponding acid chloride **147g** (0.493 g, 2.96 mmol) and aniline **148a** (0.448 g, 2.96 mmol) in a similar manner as described for preparation of **145g**, mp 93-95 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (3H, s, OCH_3), 6.63 (1H, d, *J* 15.8 Hz, CH), 7.08-7.13 (1H, m, ArH), 7.26-7.42 (3H, m, ArH), 7.56-7.60 (3H, m, ArH), 7.76 (1H, d, *J* 15.8 Hz, CH), 8.06 (1H, dd, *J* 1.2, 8.4 Hz, ArH), 8.90 (1H, dd, *J* 1.2, 8.4 Hz, ArH), 11.38 (1H, bs, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.61 (OCH_3), 115.12 (C, Ar), 120.81 (CH, Ar), 122.25 (CH), 122.78 (CH, Ar), 128.30 (2 x CH, Ar), 129.08 (2 x CH, Ar). 130.21 (CH, Ar), 131.10 (CH, Ar), 134.91 (C, Ar), 135.00 (CH, Ar), 142.08 (C, Ar), 142.49 (CH), 164.72 (C=O), 169.16` (C=O). ν_{max} (solid)/(cm^{-1}) 3263 (md), 1687 (st), 1604 (st), 1434 (st). MS *m/z* (**API-ES**): found 282 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 282.1125 $(M+H)^+$ (100%), calculated for $C_{17}H_{16}NO_3$ 282.1130.

Methyl 3-(3-phenylacryloylamino)benzoate **(145o).** This was obtained as a white solid (0.545 g, 1.91 mmol, 66%) from corresponding acid chloride **137g** (0.479 g, 2.88 mol) and aniline **148d** (0.434 g, 2.88 mmol) in a similar manner as described for preparation of **145g**, mp 151-153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (3H, s, OCH_3), 6.53 (1H, d, *J* 15.4 Hz, CH), 7.38-7.42 (4H, m, ArH), 7.44 (1H, t, *J* 7.9 Hz, ArH), 7.50 (1H, bs, NH), 7.53-7.76 (1H, m, ArH), 7.78 (1H, d, *J* 15.4 Hz, CH), 7.81 (1H, d, *J* 7.9 Hz, ArH), 8.03 (1H, bd, *J* 7.9 Hz, ArH), 8.12 (1H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.48 (OCH_3), 120.67 (CH), 121.02 (CH, Ar), 124.73 (CH, Ar), 125.66 (CH, Ar), 128.23 (2 x CH, Ar), 129.13 (2 x CH, Ar), 129.48 (CH, Ar), 130.35 (CH, Ar), 131.15 (C, Ar), 134.69 (C, Ar), 138.52 (C, Ar), 143.13 (CH), 164.43 (C=O), 166.97 (C=O). ν_{max} (solid)/(cm^{-1}) 3250 (st), 1721 (st), 1658 (st), 1618 (st), 1547 (st), 1484 (st), 1347 (st), 1276 (st), 1178 (st). MS *m/z* (**API-ES**): found

282 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 282.1129 $(M+H)^+$ (100%), calculated for $C_{17}H_{16}NO_3$ 282.1130.

***Methyl 4-(3-phenylacryloylamino)benzoate* (148p).** This was obtained as a pink solid (0.499 g, 1.77 mmol, 88%) from corresponding acid chloride **147g** (0.336, 2.02 mmol) and aniline **148e** (0.30 g, 2.02 mmol) in a similar manner as described for preparation of **145g**, mp 176-178 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (3H, s, OCH_3), 6.59 (1H, d, *J* 15.6 Hz, CH), 7.36-7.39 (3H, m, ArH), 7.49-7.52 (2H, m, ArH), 7.73 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.81 (1H, d, *J* 15.6 Hz, CH), 7.83 (1H, bs, NH), 8.02 (2H, d, *J* 8.8 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.27 (OCH_3), 119.28 (2 x CH, Ar), 120.57 (CH), 125.89 (C, Ar), 128.27 (2 x CH, Ar), 129.16 (2 x CH, Ar), 130.47 (CH, Ar), 131.12 (2 x CH, Ar), 134.60 (C, Ar), 142.55 (C, Ar), 143.52 (CH), 164.42 (C=O), 166.88 (C=O). ν_{max} (solid)/(cm^{-1}) 3383 (md), 1707 (st), 1670 (st), 1623 (st), 1606 (st), 1590 (st), 1519 (st), 1404 (st), 1275 (st). MS *m/z* (**API-ES**): found 282 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 282.1129 $(M+H)^+$ (100%), calculated for $C_{17}H_{16}NO_3$ 282.1130.

***3,N-diphenylacrylamide* (145q).** This was obtained as a white solid (1.45 g, 6.48 mmol, 98%) from corresponding acid chloride **147g** (1.10 g, 6.60 mmol) and aniline **148f** (0.614 g, 6.60 mmol) in a similar manner as described for preparation of **145g**, mp 148-150 °C (lit[421] 154-156 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.55 (1H, d, *J* 15.4 Hz, CH), 7.14 (1H, t, *J* 7.6 Hz, ArH), 7.48 (6H, m, ArH), 7.53 (2H, m, ArH), 7.62 (2H, bd, *J* 7.6 Hz, ArH), 7.76 (1H, d, *J* 15.4 Hz, CH).

***Methyl 4-(3-phenylacryloylamino)benzoate* (145r).** This was obtained as a white solid from corresponding acid chloride **147g** (1.1 g, 6.62 mmol) and aniline **148b** (1.10 g, 6.62 mmol) in a similar manner as described for preparation of **145g**. The crude was used in the next step without further purification.

***Methyl 4-[2-(2-nitrophenylcarbamoyl)vinyl]benzoate* (145s).** This was obtained as a yellow solid (0.189 g, 0.579 mmol, 93%) from corresponding acid chloride **147a** (0.140 g, 0.625 mmol) and aniline **148g** (0.095 g, 0.688 mmol) in a similar manner as described for preparation of **145g**, mp173-175 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (3H, s, OCH_3), 6.64 (1H, d, *J* 15.6 Hz, CH), 7.14-7.18 (1H. m, ArH), 7.59 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.62-7.66 (1H, s, ArH), 7.72 (2H, d, *J* 15.6 Hz, CH), 8.02 (1H, d, *J* 8.4 Hz, 2 x CH, Ar), 8.20 (1H, dd, *J* 1.3, 8.5 Hz, ArH), 8.88 (1H, dd, *J* 1.3, 8.5 Hz, ArH), 10.62 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.45 (OCH_3), 116.21 (C, Ar), 122.14 (CH, Ar), 123.12 (CH), 124.48 (CH, Ar), 129.04 (CH, Ar), 130.77 (CH, Ar), 131.12 (CH, Ar), 133.56 (C, Ar), 135.32 (CH, Ar), 140.05 (C, Ar), 141.47 (CH), 141.98 (C, Ar), 166,75 (C=O), 170.05 (C=O). ν_{max} (solid)/(cm^{-1}) 3370 (st), 1711 (st), 1688 (st), 1605 (st), 1581 (st), 1494 (st), 1340 (st), 1316

(st), 1268 (st), 1105 (st). MS *m/z* (**API-ES**): found 327 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 327.0961 $(M+H)^+$, calculated for $C_{17}H_{15}N_2O_5$ 327.0981; found 189.0539 $(M-C_8H_8NO_2)^+$, calculated for $C_{11}H_9O_3$ 189.0552; found $(M+Na)^+$ 349.0787, calculated for $C_{17}H_{14}N_2O_5Na$ 349.0800; found 675.1683 $(2M+Na)^+$, calculated for $C_{34}H_{28}N_4O_{10}Na$ 675.1703.

Methyl 2-(4-nitro-3-phenylbutyrylamino)benzoate (144l)

A mixture of **145n** (0.227 g, 0.796 mmol), DBU (0.145 g, 0.955 mmol) in nitromethane (5 mL), was stirred in the microwave reactor at 100 °C for 15 min. After cooling to room temperature, the reaction mixture was poured into HCl (aq, 1M, 10 ml). The product extracted with ethyl acetate (2 x 15 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure. Chromatography on silica gel using the FlashMaster 3 purification station (80:20 hexanes/ethyl acetate, R_f 0.20) afforded **144l** (0.140 g, 0.460 mmol, 51%) as off-white solid, mp 104-106 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.85 (1H, dd, *J* 7.2, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 2.91 (1H, dd, *J* 7.2, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 3.98 (3H, m, OCH_3), 4.13 (1H, quint, *J* 7.3 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.72 (1H, dd, *J* 8.4, 12.7 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 4.86 (1H, dd, *J* 6.6, 12.7 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 7.06-7.10 (1H, m, ArH), 7.23-7.34 (5H, m, ArH), 7.49-7.54 (1H, m, ArH), 8.00 (1H, dd, *J* 1.4, 8.3 Hz, ArH), 8.00 (1H, d, *J* 8.3 Hz, ArH), 11.12 (1H, bs, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.64 (CH_2, $NO_2CH_2CH\underline{C}H_2CO$), 41.71 (CH, $NO_2CH_2\underline{C}HCH_2CO$), 52.63 ($OCH_3$), 76.64 ($CH_2$, $NO_2\underline{C}H_2CHCH_2CO$), 115.20 (C, Ar), 120.63 (CH, Ar), 123.07 (CH, Ar), 127.61 (2 x CH, Ar), 128.17 (2 x CH, Ar), 129.31 (CH, Ar), 131.04 (CH, Ar), 134.92 (CH, Ar), 138.76 (C, Ar), 141.21 (C, Ar), 168.77 (C=O), 168.93 (C=O). ν_{max} (solid)/(cm^{-1}) 3268 (st), 1684 (st), 1544 (st), 1448 (md), 1428 (md), 1258 (st). MS *m/z* (**API-ES**): found 343 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 343.1294 $(M+H)^+$ (100%), calculated for $C_{18}H_{19}N_2O_5$ 343.1294.

Ethyl 4-(4-nitro-3-phenylbutyrylamino)benzoate **(144a)**. This was prepared from corresponding amide **145r** (1.340 g, 4.44 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel (7:3 hexanes/ethyl acetate, R_f 0.20) afforded **144a** (0.980 g, 2.75 mmol, 62%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, *J* 7.4 Hz, $CH_2C\underline{H_3}$), 2.79 (1H, dd, *J* 7.2, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 2.86 (1H, dd, *J* 7.2, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 4.07 (1H, quint, *J* 7.2 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.34 (2H, q, *J* 7.4 Hz, $C\underline{H_2}CH_3$), 4.72 (1H, dd, *J* 7.8, 12.6 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 4.83 (1H, dd, *J* 6.4, 12.4 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 7.21 (1H, bs, NH), 7.22-7.30 (2H, m, ArH), 7.27-7.36 (3H, m, ArH), 7.47 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.96 (2H, d, *J* 8.8 Hz, 2 x CH, Ar). ^{13}C NMR (400 MHz, $CDCl_3$) δ 14.55 ($CH_2\underline{C}H_3$), 40.67 (CH_2, $NO_2CH_2CH\underline{C}H_2CO$), 40.93 ($CH_2$, $NO_2CH_2\underline{C}HCH_2CO$), 61.20 ($\underline{C}H_2CH_3$), 79.50 ($NO_2\underline{C}H_2CHCH_2CO$), 119.17 (2 x CH, Ar), 126.54 (C, Ar), 127.53 (2 x CH, Ar), 128.45 (CH, Ar), 129.48 (2 x CH, Ar), 130.98 (2 x CH, Ar), 138.51 (C, Ar), 141.60 (C, Ar),

166.30 (C=O), 168.39 (C=O). ν_{max} (solid)/(cm^{-1}) 3254 (st), 1664 (st), 1535 (st), 1426 (md), 1236 (st). MS *m/z* (**API-ES**): found 357 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 357.1344 $(M+H)^+$ (100%), calculated for $C_{19}H_{21}N_2O_5$ 357.1450.

Ethyl 4-(3-naphthalen-2-yl-4-nitrobutyrylamino)benzoate **(144b)**. This was prepared from corresponding amide **145b** (0.995 g, 2.88 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using the FlashMaster 3 purification station (75:25 hexanes/ethyl acetate, R_f 0.20) afforded **144b** (0.819 g, 2.017 mmol, 70%) as a yellow oil. ^1H NMR (400 MHz, DMSO-d_6) δ 1.26 (3H, t, *J* 7.4 Hz, $CH_2C\underline{H}_3$), 2.85-2.96 (2H, m, 2 x CH, $NO_2CH_2CHC\underline{H}_2CO$), 4.10 (1H, quint, *J* 8.0 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.23 (1H, q, *J* 7.4 Hz, $C\underline{H}_2CH_3$), 5.04 (1H, dd, *J* 9.0, 13.0 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 5.12 (1H, dd, *J* 5.2, 12.0, Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 7.45-7.48 (2H, m, ArH), 7.54 (1H, dd, *J* 1.6, 8.8 Hz, ArH), 7.62 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.82-7.87 (6H, m, ArH) 10.33 (1H, bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) δ 14.52 ($CH_2\underline{C}H_3$), 40.75 ($NO_2CH_2\underline{C}HCH_2CO$), 40.79 ($NO_2CH_2CH\underline{C}H_2CO$), 61.21 ($\underline{C}H_2CH_3$), 79.48 ($NO_2\underline{C}H_2CHCH_2CO$), 119.21 (2 x CH, Ar), 124.97 (CH, Ar), 126.43 (CH, Ar), 126.65 (C, Ar), 126.78 (CH, Ar), 126.87 (CH, Ar), 127.92 (CH, Ar), 128.04 (CH, Ar), 129.37 (CH, Ar), 130.94 (2 x CH, Ar), 133.11 (C, Ar), 133.59 (C, Ar), 135.89 (C, Ar), 141.68 (C, Ar), 166.37 (C=O), 168.54 (C=O). ν_{max} (solid)/(cm^{-1}) 3255 (st), 1676 (st), 1539 (st), 1365 (md), 1245 (st). MS *m/z* (**API-ES**): found 407 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 407.1607 $(M+H)^+$ (100%), calculated for $C_{23}H_{23}N_3O_7$ 407.1607.

Methyl 2-hydroxy-4-(3-naphthalen-2-yl-4-nitro-3-butyrylamino)benzoate **(144c)**. This was prepared from corresponding amide **145c** (0.750 g, 2.16 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel (70:30 hexanes/ethyl acetate, R_f 0.22) afforded **144c** (0.467 g, 1.14 mmol, 55%) as a white solid, 145-147 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 2.86 (1H, dd, *J* 7.6, 15.6 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 2.92 (1H, dd, *J* 6.4, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 3.81 (3H, s, OCH_3), 4.09 (1H, quint, *J* 7.6 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 5.04 (1H, dd, *J* 9.2, 13.2 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 5.09 (1H, dd, *J* 5.8, 13.0 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 6.97 (1H, dd, *J* 1.8, 8.8 Hz, H-2), 7.28 (1H, d, *J* 1.8 Hz, H-6), 7.46-749 (2H, m, ArH), 7.53 (1H, dd, *J* 1.6, 8.4 Hz, ArH), 7.65 (1H, d, *J* 8.8 Hz, H-3), 7.81-7.87 (4H, m, ArH), 10.27 (1H, bs, NH), 10.55 (1H, s, OH). ^{13}C NMR (400 MHz, DMSO-d_6) δ 40.35 ($NO_2CH_2\underline{C}HCH_2CO$), 40.84 ($NO_2CH_2CH\underline{C}H_2CO$), 52.87 ($OCH_3$), 80.16 ($NO_2\underline{C}H_2CHCH_2CO$), 106.79 (CH, Ar), 108.15 (C, Ar), 111.06 (CH, Ar), 126.37 (CH, Ar), 127.66 (CH, Ar), 126.97 (CH, Ar), 127.12 (CH, Ar), 128.18 (CH, Ar), 128.24 (CH, Ar), 128.83 (CH, Ar), 131.44 (CH, Ar), 132.94 (C, Ar), 133.54 (C, Ar), 137.83 (C, Ar), 145.90 (C, Ar), 161.81 (C, Ar), 169.61 (C=O), 169.98 (C=O). ν_{max} (solid)/(cm^{-1}) 3380 (md), 1698 (st), 1667 (st), 1597 (st), 1544 (st), 1541 (st), 1190 (st), 1151

(st). MS *m/z* (**API-ES**): found 409 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 409.1402 $(M+H)^+$, calculated for $C_{22}H_{21}N_2O_6$ 409.1400.

***Methyl 2-[3-(4-chlorophenyl)-4-nitrobutyrylamino]benzoate* (144e).** This was prepared from the corresponding amide **145g** (0.209 g, 0.680 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed by the Flash Master 3 purification station (80:20 hexanes/ethyl acetate, R_f 0.26) afforded **144e** (0.088 g, 0.234 mmol, 35%) as an off-white solid, mp 105-107 °C. ^{1}H NMR 400 MHz, ($CDCl_3$) δ 2.84 (1H, dd, *J* 7.4, 14.6 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 2.89 (1H, dd, *J* 7.4, 14.6 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 3.91 (3H, s, OCH_3), 4.12 (1H, quint, *J* 7.3 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.69 (1H, dd, *J* 8.8, 12.7 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 4.84 (1H, dd, *J* 6.2, 12.7 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 7.07-7.11 (1H, m, ArH), 7.22 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.30 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.50-7.54 (1H, m, ArH), 8.01 (1H, dd, *J* 1.4, 8.3 Hz, ArH), 8.59 (1H, d, *J* 8.3 Hz, ArH), 11.12 (1H, bs, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.03 (CH_2, $NO_2CH_2C\underline{H}C\underline{H}_2CO$), 41.55 (CH, $NO_2CH_2C\underline{H}C\underline{H}_2CO$), 52.65 ($OCH_3$), 79.39 ($CH_2$, $NO_2\underline{C}H_2CHCH_2CO$), 115.20 (C, Ar), 120.62 (CH, Ar), 123.17 (CH, Ar), 129.05 (2 x CH, Ar), 129.50 (2 x CH, Ar), 131.08 (CH, Ar), 134.06 (C, Ar), 134.95 (CH, Ar), 137.24 (C, Ar), 141.13 (C, Ar), 168.34 (C=O), 168.97 (C=O). ν_{max} (solid)/(cm^{-1}) 3275 (md), 1691(st), 1545 (st), 1524 (st), 1447 (md), 1430 (md), 1268 (md), 1256 (st). MS *m/z* (**API-ES**): found 377 $(M^{35}Cl+H)^+$ (100%), 379 $(M^{37}Cl+H)^+$ (40%). HRMS *m/z* (**API-ES**): found 377.0909 $(M+H)^+$ (100%), calculated for $C_{18}H_{18}ClN_2O_5$ 377.0904.

***Methyl 3-[3-(4-chlorophenyl)-4-nitrobutyrylamino]benzoate* (144f).** This was prepared from corresponding amide **145h** (0.160 g, 0.524 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using the FlashMaster 3 purification station (70:30 hexanes/ethyl acetate) afforded **144f** (0.136 g, 0.400 mmol, 77%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.77 (1H, dd, *J* 7.6, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 2.85 (1H, dd, *J* 7.2, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 3.88 (3H, s, OCH_3), 4.08 (1H, quint, *J* 7.8 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.69 (1H, dd, *J* 8.0, 12.6 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 4.81 (1H, dd, *J* 6.6, 12.6 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 7.16 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.27 (2H, d, *J* 8.6 Hz, 2 x CH, Ar) 7.36 (1H, t, *J* 7.6 Hz, ArH), 7.76 (2H, d, *J* 8.4 Hz, ArH), 7.87 (1H, s, NH), 7.94 (1H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.07 (CH_2, $NO_2CH_2C\underline{H}C\underline{H}_2CO$), 40.36 (CH, $NO_2CH_2\underline{C}HCH_2CO$), 52.67 ($OCH_3$), 79.39 ($CH_2$, $NO_2\underline{C}H_2CHCH_2CO$), 121.31 (CH, Ar), 125.09 (CH, Ar), 126.06 (CH, Ar), 128,98 (2 x CH, Ar), 129.48 (2 x CH, Ar), 129.53 (CH, Ar), 130.98 (C, Ar), 134.17 (C, Ar), 137.10 (C, Ar), 137.73 (C, Ar), 167.19 (C=O), 168.81 (C=O). ν_{max} (oil)/(cm^{-1}) 3326 (md), 2954 (st), 2924 (st), 1716 (st), 1670 (st), 1547 (st), 1431 (st), 1291 (st). MS *m/z* (**API-ES**): found 394 $(M^{35}Cl+NH_4)^+$ (100%), 377 $(M^{35}Cl+H)^+$ (5%). HRMS *m/z* (**API-ES**): found $(M+H)^+$

377.0903, calculated for $C_{18}H_{18}ClN_2O_5$ 377.0904; found $(M+NH_4)^+$ 394.1174, calculated for $C_{18}H_{21}ClN_3O_5$ 394.1170.

Methyl 4-[3-(4-chlorophenyl)-4-nitrobutyrylamino]benzoate **(144g)**. This was prepared from corresponding amide **145i** (0.152 g, 0.498 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed by the Flash Master 3 purification station (70:30 hexanes/ethyl acetate, R_f 0.16) afforded **144g** (0.131 g, 0.350 mmol, 71%) as an off-white solid, mp 114-116 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.76 (1H, dd, *J* 7.1, 15.5 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 2.83 (1H, dd, *J* 7.1, 15.5 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 3.88 (3H, s, OCH_3), 4.06 (1H, quint, *J* 7.2 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.67 (1H, dd, *J* 8.0, 12.7 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 4.81 (1H, dd, *J* 6.6, 12.7 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 7.15 (2H, d, *J* 8.2 Hz, 2 x CH, Ar), 7.27 (2H, d, *J* 8.2 Hz, 2 x CH, Ar), 7.50 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.92 (1H, bs, NH), 7.94 (2H, t, *J* 8.6 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.95 (CH_2, $NO_2CH_2CH\underline{C}H_2CO$), 40.45 (CH, $NO_2CH_2\underline{C}HCH_2CO$), 52.37 ($OCH_3$), 79.37 (CH, $NO_2\underline{C}H_2CHCH_2CO$), 119.31 (2 x CH, Ar), 126.11 (C, Ar), 128.94 (2 x CH, Ar), 129.57 (2 x CH, Ar), 131.03 (2 x CH, Ar), 134.21 (C, Ar), 137.10 (C, Ar), 141.84 (C, Ar), 166.91 (C=O), 168.37 (C=O). ν_{max} (solid)/(cm^{-1}) 3340 (md), 1700 (st), 1669 (st), 1553 (st), 1514 (st), 1407 (md), 1280 (st). MS *m/z* (**API-ES**): found 377 $(M^{35}Cl+H)^+$ (100%), 379 $(M^{37}Cl+H)^+$ (35%). HRMS *m/z* (**API-ES**): found 377.0902 $(M+H)^+$ (100%), calculated for $C_{18}H_{18}ClN_2O_5$ 377.0904.

Methyl 2-[3-(4-methoxyphenyl)-4-nitrobutyrylamino]benzoate **(144h)**. This was prepared from corresponding amide **145j** (0.176 g, 0.565 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed by the Flash Master 3 purification station (80:20 hexanes/ethyl acetate, R_f 0.20) afforded **144h** (0.084 g, 0.226 mmol, 40%) as a off-white solid, 81-83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.82 (1H, dd, *J* 7.4, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 2.88 (1H, dd, *J* 7.4, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 3.76 (3H, m, OCH_3), 3.19 (3H, s, OCH_3), 4.08 (1H, quint, *J* 7.3 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.67 (1H, dd, *J* 8.4, 12.5 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 4.82 (1H, dd, *J* 6.6, 12.5 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 6.84 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.06-7.10 (1H, m, ArH), 7.19 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.50-7.54 (1H, m, ArH), 8.00 (1H, dd, *J* 1.2, 8.2 Hz, ArH), 8.61 (1H, dd, *J* 1.2, 8.2 Hz, ArH), 11.10 (1H, bs, NH).^{13}C NMR (100 MHz, $CDCl_3$) δ 40.02 (CH_2, $NO_2CH_2CH\underline{C}H_2CO$), 41.93 (CH, $NO_2CH_2\underline{C}HCH_2CO$), 52.62 ($OCH_3$), 55.44 ($OCH_3$), 79.91 ($CH_2$, $NO_2\underline{C}H_2CHCH_2CO$), 114.67 (2 x CH, Ar), 115.22 (C, Ar), 120.65 (CH, Ar), 123.08 (CH, Ar), 128.68 (2 x CH, Ar), 130.59 (C, Ar), 131.03 (CH, Ar), 134.90 (CH, Ar), 141.20 (C, Ar), 159.37 (C=O), 168.93 (C=O). ν_{max} (solid)/(cm^{-1}) 3272 (md), 1698 (st), 1681 (st), 1603 (md), 1687 (md), 1545 (st), 1514 (st), 1447 (md), 1431 (md), 1250 (st). MS *m/z* (**API-ES**): found 373 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 373.1399 $(M+H)^+$ (100 %), calculated for $C_{19}H_{21}N_2O_6$ 373.1400.

Methyl 3-[3-(4-methoxyphenyl)-4-nitrobutyrylamino]benzoate **(144i)**. This was prepared from corresponding amide **145k** (0.156 g, 0.500 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed performed using the FlashMaster 3 purification station (80:20 hexanes/ethyl acetate) afforded **144i** (0.129 g, 0.346 mmol, 69%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 (1H, dd, *J* 7.2, 15.2 Hz, CH, $NO_2CH_2CHCH_2CO$), 2.82 (1H, dd, *J* 7.4, 15.0 Hz, CH, $NO_2CH_2CHCH_2CO$), 3.74 (3H, s, OCH_3), 3.86 (3H, s), 4.03 (1H, quint, *J* 7.3 Hz, CH, $NO_2CH_2CHCH_2CO$), 4.65 (1H, dd, *J* 7.8, 12.6 Hz, CH, $NO_2CH_2CHCH_2CO$), 4.77 (1H, dd, *J* 6.8, 12.4 Hz, CH, $NO_2CH_2CHCH_2CO$), 6.81 (2H, d, *J* 8.6 Hz, 2 x CH, ArH), 7.12 (2H, d, *J* 8.6 Hz, 2 x CH, ArH) 7.33 (1H, t, *J* 7.6 Hz, ArH), 7.74 (2H, d, *J* 7.6 Hz, ArH), 7.94-7.95 (2H, m, CH, ArH, & NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.07 (CH_2, $NO_2CH_2CHCH_2CO$), 40.75 (CH, $NO_2CH_2CHCH_2CO$), 52.55 (OCH_3), 55.45 (OCH_3), 79.90 (CH_2, $NO_2CH_2CHCH_2CO$), 114.71 (2 x CH, Ar), 121.22 (CH, Ar), 124.95 (CH, Ar), 125.81 (CH, Ar), 128.62 (2 x CH, Ar), 129.36 (CH, Ar), 130.44 (C, Ar), 130.96 (C, Ar), 137.97 (C, Ar), 159.43 (C, Ar), 167.06 (C=O), 169.07 (C=O). ν_{max} (solid)/(cm^{-1}) 3323 (md), 1718 (st), 1668 (st), 1547 (st), 1513 (st), 1439 (st), 1297 (st), 1249 (st). MS *m/z* (**API-ES**): found 390 $(M+NH_4)^+$ (100%), 373 $(M+H)^+$ (40%). HRMS *m/z* (**API-ES**): found 373.1397 $(M+H)^+$, calculated for $C_{19}H_{21}N_2O_6$ 373.1400; found 390.1675 $(M+NH_4)^+$, calculated for $C_{19}H_{24}N_3O_6$ 390.1665.

Methyl 4-[3-(4-methoxyphenyl)-4-nitrobutyrylamino]benzoate **(144j)**. This was prepared from corresponding amide **145l** (0.189 g, 0.607 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using the FlashMaster 3 purification station (70:30 hexanes/ethyl acetate, R_f 0.15) to give **144j** (0.146 g, 0.400 mmol, 57%) as an off-white solid, mp 89-91 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 (1H, dd, *J* 6.9, 15.1 Hz, CH, $NO_2CH_2CHCH_2CO$), 2.84 (1H, dd, *J* 6.9, 15.1 Hz, CH, $NO_2CH_2CHCH_2CO$), 3.76 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.06 (1H, quint, *J* 7.3 Hz, CH, $NO_2CH_2CHCH_2CO$), 4.67 (1H, dd, *J* 7.6, 12.4 Hz, CH, $NO_2CH_2CHCH_2CO$), 4.76 (1H, dd, *J* 6.4, 12.4 Hz, CH, $NO_2CH_2CHCH_2CO$), 6.85 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.15 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.48 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.49 (1H, bs, NH), 7.96 (2H, d, *J* 8.6 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.00 (CH_2, $NO_2CH_2CHCH_2CO$), 40.90 (CH, $NO_2CH_2CHCH_2CO$), 52.33 (OCH_3), 55.47 (OCH_3), 79.87 (CH_2, $NO_2CH_2CHCH_2CO$), 114.78 (2 x CH, Ar), 119.30 (2 x CH, Ar), 126.00 (C, Ar), 128.59 (2 x CH, Ar), 130.35 (C, Ar), 130.98 (2 x CH, Ar), 141.95 (C, Ar), 159.48 (C, Ar), 166.90 (C=O), 168.83 (C=O). ν_{max} (solid)/(cm^{-1}) 3327 (md), 1707 (st), 1658 (st), 1548 (st), 1516 (st), 1279 (st). MS *m/z* (**API-ES**): found 473 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 373.1404 $(M+H)^+$ (100%), calculated for $C_{19}H_{21}N_2O_6$ 373.1400.

Methyl 2-[4-nitro-3-(3,4,5-trimethoxyphenyl)butyrylamino]benzoate **(144k)**. This was prepared from corresponding amide **145m** (0.120 g, .0323 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using FlashMaster 3 purification station (60:40 hexanes/ethyl acetate) afforded **144k** (0.048 g, 0.111mmol, 34%) as an off white solid, 130-132 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81 (1H, dd, *J* 15.0, 7.4 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 2.86 (1H, dd, *J* 15.2, 7.2 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 3.76 (3H, s, OCH_3), 3.80 (6H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.01-4.11 (1H, m, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.71 (1H, dd, *J* 12.8, 8.4 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 4.82 (1H, dd, *J* 12.6, 6.6 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 6.44 (2H, s, H-2' & H-6'), 7.05-7.09 (1H, m, ArH), 7.49 (1H, m, ArH), 7.98 (1H, dd, *J* 1.4, 7.9 Hz), 8.60 (1H, d, *J* 7.9 Hz), 11.08 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.15 ($NO_2CH_2\underline{C}HCH_2CO$), 42.07 ($NO_2CH_2CH\underline{C}H_2CO$), 52.61 ($OCH_3$), 56.21 (2 x OCH_3), 60.96 (OCH_3), 79.54 ($NO_2\underline{C}H_2CHCH_2CO$), 104.53 (2 x CH, Ar), 115.11 (C, Ar), 120.50 (CH, Ar), 123.11 (CH, Ar), 131.08 (CH, Ar), 134.17, 134.91 (CH, Ar), 137.73 (C, Ar), 141.18 (C, Ar), 153.75 (C, Ar), 168.73 (C=O), 168.90 (C=O). ν_{max} (solid)/(cm^{-1}) 3265 (md), 1702 (st), 1680 (st), 1589 (st), 1680 (st), 1589 (st), 1541 (st), 1448 (st), 1429 (st), 1259 (st), 1238 (st), 1123 (st). MS *m/z* (**API-ES**): found 433 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 433.1598 $(M+H)^+$, calculated for $C_{21}H_{25}N_2O_8$ 433.1611; found 887.2939 $(2M+Na)^+$, calculated for $C_{42}H_{48}N_4O_{16}Na$ 887.2963.

Methyl 3-(4-nitro-3-phenylbutyrylamino)benzoate **(144m)**. This was prepared from corresponding amide **145o** (0.148 g, 0.519 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using the FlashMaster 3 purification station (70:30 hexanes/ethyl acetate) afforded **144m** (0.126 g, 0.368 mmol, 70%) as an off-white solid, mp 103-105 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.78 (1H, dd, *J* 7.6, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 2.84 (1H, dd, *J* 7.4, 15.7 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 3.86 (3H, s, OCH_3), 4.06 (1H, quint, *J* 7.4 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.69 (1H, dd, *J* 8.0, 12.4 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 4.81 (1H, dd, *J* 6.4, 12.8 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 7.29-7.35 (6H, m, 5 x CH, Ar, & NH), 7.73 (2H, t, *J* 8.0 Hz, 2 x CH, Ar), 7.92 (2H, s, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.64 (CH_2, $NO_2CH_2CH\underline{C}H_2CO$), 40.73 (CH, $NO_2CH_2\underline{C}HCH_2CO$), 52.59 ($OCH_3$), 79.61 ($CH_2$, $NO_2\underline{C}H_2CHCH_2CO$), 121.26 (CH, Ar), 125.01 (CH, Ar), 125.91 (CH, Ar), 127.54 (2 x CH, Ar), 128.34 (CH, Ar), 129.05 (2 x CH, Ar), 130.98 (C, Ar), 137.85 (C, Ar), 138.60 (C, Ar), 167.04 (C=O), 168.95 (C=O). ν_{max} (solid)/(cm^{-1}) 3337 (md), 1704 (st), 1683 (st), 1591 (st), 1431 (st). MS *m/z* (**API-ES**): found 360 $(M+NH_4)^+$ (100%), 343 $(M+H)^+$(30%). HRMS *m/z* (**API-ES**): found 343.12942 $(M+H)^+$, calculated for $C_{18}H_{19}N_2O_5$ 343.1294; found $(M+NH_4)^+$ 360.1564, calculated for $C_{18}H_{22}N_3O_5$ 360.1559.

Methyl 4-(4-nitro-3-phenylbutyrylamino)benzoate **(145n)**. This was prepared from corresponding amide **145p** (0.234, 0.832 mmol) in a similar manner as described for

preparation of **144l**. Chromatography on silica gel performed by the FlashMaster 3 purification station (80:20 hexanes/ethyl acetate, R_f 0.17) afforded **144n** (0.231 g, 0.675 mmol, 81%) as an off-white solid, mp 93-95 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 (1H, dd, *J* 7.0, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 2.88 (1H, dd, *J* 7.8, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 3.89 (3H, s, OCH_3), 4.08 (1H, quint, *J* 7.2 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.73 (1H, dd, *J* 7.6, 12.5 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 4.85 (1H, dd, *J* 6.6, 12.5 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 7.25 (H, d, *J* 7.2 Hz, CH, Ar), 7.28-7.37 (H, m, CH, Ar), 7.40 (1H, bs, NH), 7.47 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.94 (2H, t, *J* 8.8 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.67 (CH_2, $NO_2CH_2CH\underline{C}H_2CO$), 40.91 (CH, $NO_2CH_2\underline{C}HCH_2CO$), 52.31 ($OCH_3$), 79.51 ($CH_2$, $NO_2\underline{C}H_2CHCH_2CO$), 119.23 (2 x CH, Ar), 126.18 (C, Ar), 127.53 (2 x CH, Ar), 128.45 (CH, Ar), 129.48 (2 x CH, Ar), 131.03 (2 x CH, Ar), 138.50 (C, Ar), 141.70 (C, Ar), 166.77 (C=O), 168.45 (C=O). ν_{max} (solid)/(cm^{-1}). 3360 (st), 1707 (st), 1671 (st), 1595 (md), 1541 (st), 1513 (st), 1273 (st), 1246 (md). MS *m/z* (**API-ES**): found 343 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 343.1298 $(M+H)^+$, calculated for $C_{18}H_{19}N_2O_5$ 343.1294.

Methyl 2-[5-nitro-3-(4-nitrophenyl)pentanoylamino]benzoate **(149a)**. This was prepared from corresponding amide **145d** (0.246 g, 0.754 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed by the Flash Master 3 purification station (80:20 hexanes/ethyl acetate, R_f 0.18) afforded **149a** (0.087 g, 0.217 mmol, 29%) as an off-white solid, mp 80-82 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34-2.40 (1H, m, CH, $NO_2CH_2C\underline{H}_2CHCH_2$), 2.59-2.64 (1H, m, CH, $NO_2CH_2C\underline{H}_2CHCH_2$), 2.79 (1H, dd, *J* 7.6, 14.8 Hz, CH, $NO_2CH_2CH_2CHC\underline{H}_2$), 2.86 (1H, dd, *J* 6.8, 15.2 Hz, CH, $NO_2CH_2CH_2CHC\underline{H}_2$), 3.48-3.55 (1H, m, CH, $NO_2CH_2CH_2C\underline{H}CH_2$), 3.90 (3H, s, OCH_3), 4.23-4.28 (2H, m, 2 x CH, $NO_2C\underline{H}_2CH_2CHCH_2$), 7.06-7.10 (1H, m, ArH), 7.46 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.49-7.53 (1H, m, ArH), 7.99 (1H, dd, *J* 1.1, 8.18 Hz, ArH), 8.19 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 8.56 (1H, dd, *J* 1.1, 8.1 H, ArH), 11.09 (1H, bs, NH), mp 80-82 °C. ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.99 (CH_2), 39.52 ($NO_2CH_2CH_2\underline{C}HCH_2$), 44.99 ($CH_2$), 52.65 ($OCH_3$), 73.41 ($NO_2\underline{C}H_2CH_2CHCH_2$), 115.11 (C, Ar), 120.55 (CH, Ar), 123.16 (CH, Ar), 124.51 (2 x CH, Ar), 128.73 (2 x CH, Ar), 131.10 (CH, Ar), 134.98 (CH, Ar), 141.12 (C, Ar), 147.52 (C, Ar), 149.26 (C, Ar), 168.58 (C=O), 169.02 (C=O). ν_{max} (solid)/(cm^{-1}) 3260 (md), 1689 (st), 1673 (st), 1589 (st), 1549 (st), 1516 (st), 1449 (st), 1431 (st), 1345 (st), 1315 (st), 1263 (st), 1238 (st). MS *m/z* (**API-ES**): found 402 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 402.1307 $(M+H)^+$, calculated for $C_{19}H_{20}N_3O_7$ 402.1301.

Methyl 2-[4-nitro-3-(4-nitrophenyl)pentanoylamino]benzoate **(149b)**. This was prepared from corresponding amide **145d** (0.246 g, 0.754 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed by the FlashMaster 3 purification station (80:20 hexanes/ethyl acetate, R_f 0.27) afforded **149b** (0.055 g, 0.137

mmol, 18%) as a off-white solid, mp 74-76 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29-2.38 (1H, m, CH, $NO_2CH_2C\underline{H_2}CHCO$), 2.43-2.52 (1H, m, CH, $NO_2CH_2C\underline{H_2}CHCO$), 2.75-2.83 (1H, m, CH, $NO_2CH_2CH_2C\underline{H}CO$), 3.00 (1H, dd, *J* 5.6, 13.6 Hz, CH, $NO_2CH_2CH_2C\underline{H_2}$), 3.17 (1H, dd, *J* 9.0, 14.4 Hz, CH, $NO_2CH_2CH_2C\underline{H_2}$), 3.85 (3H, m, OCH_3), 4.42-4.57 (2H, m, 2 x CH, $NO_2C\underline{H_2}CH_2CHCO$), 7.09-7.13 (1H, m, ArH), 7.37 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.52-7.56 (1H, m, ArH), 7.98 (1H, dd, *J* 1.3, 8.3 Hz, ArH), 8.10 (2H, d, *J* 8.6 Hz, 2 x CH, ArH), 8.53 (1H, dd, *J* 1.3, 8.3 Hz, ArH), 11.04 (1H, bs, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.85 (CH_2), 39.22 (CH_2), 48.07 ($NO_2CH_2CH_2\underline{C}HCO$), 52.66 ($OCH_3$), 73.32 ($NO_2\underline{C}H_2CH_2CHCO$), 115.39 (C, Ar), 120.62 (CH, Ar), 123.54 (CH, Ar), 124.10 (2 x CH, Ar), 130.09 (2 x CH, Ar), 131.17 (CH, Ar), 134.92 (CH, Ar), 140.61 (C, Ar), 145.92 (C, Ar), 147.20 (C, Ar), 168.71 (C=O), 171.27 (C=O). ν_{max} (solid)/(cm^{-1}) 3237 (md), 1684 (st), 1670 (st), 1606 (st), 1589 (st), 1551 (st), 1517 (st), 1446 (st), 1429 (st), 1344 (st), 1267 (st). MS *m/z* (**API-ES**): found 402 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 402.1302 $(M+H)^+$, calculated for $C_{19}H_{20}N_3O_7$ 402.1301.

Methyl 3-[5-nitro-3-(4-nitrophenyl)pentanoylamino]benzoate **(150a)**. This was prepared from corresponding amide **145e** (0.151 g, 0.463 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using the FlashMaster 3 purification station (70:30 hexanes/ethyl acetate) afforded **150a** (0.023 g, 0.057 mmol, 12%) as a yellow solid, mp 125-127 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.31-2.40 (1H, m, CH, $NO_2CH_2C\underline{H_2}CHCH_2CO$), 2.57-2.66 (1H, m, CH, $NO_2CH_2C\underline{H_2}CHCH_2CO$), 2.72 (1H, dd, *J* 8.0, 15.2 Hz, CH, $NO_2CH_2CH_2CHC\underline{H_2}CO$), 2.81 (1H, dd, *J* 6.8, 15.2 Hz, CH, $NO_2CH_2CH_2CHC\underline{H_2}CO$), 3.44-3.55 (1H, m, CH, $NO_2CH_2CH_2C\underline{H}CH_2CO$), 3.89 (3H, s, OCH_3), 4.25 (2H, t, *J* 7.6 Hz, CH_2, $NO_2C\underline{H_2}CH_2CHCH_2CO$), 7.36 (1H, t, *J* 7.6 Hz, ArH), 7.42 (2H, d, *J* 8.8 Hz, 2 x CH, Ar, 7.56 (1H, s, ArH), 7.74-7.77 (2H, m, ArH), 7.92 (1H, bs, NH), 8.18 (2H, d, *J* 8.4 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.86 (CH_2), 39.44 ($NO_2CH_2CH_2\underline{C}HCH_2CO$), 43.85 ($CH_2$), 52.57 ($OCH_3$), 73.41 ($NO_2\underline{C}H_2CH_2CHCH_2CO$), 121.03 (CH, Ar), 124.56 (2 x CH, Ar), 124.76 (CH, Ar), 125.98 (CH, Ar), 128.70 (2 x CH, Ar), 129.50 (CH, Ar), 131.14 (C, Ar), 137.70 (C, Ar), 147.55 (C, Ar), 149.22 (C, Ar), 166.86 (C=O), 168.34 (C=O). ν_{max} (solid)/(cm^{-1}) 1672 (st), 1589 (st), 1549 (st), 1516 (st), 1345 (st), 1315 (st), 1263 (st), 1088 (st), 858 (st), 760 (st), 697 (st). MS *m/z* (**API-ES**): found 419 $(M+NH_4)^+$ (100 %). HRMS *m/z* (**API-ES**): found 402.1295 $(M+H)^+$, calculated for $C_{19}H_{20}N_3O_7$ 402.1301; found 419.1561 $(M+NH_4)^+$, calculated for $C_{19}H_{23}N_4O_7$ 419.1567.

Methyl 3-[4-nitro-2-(4-nitrobenzyl)butyrylamino]benzoate **(150b)**. This was prepared from corresponding amide **145e** (0.151 g, 0.463 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using the FlashMaster 3 purification station (70:30 hexanes/ethyl acetate) afforded **140b** (0.066 g, 0.164 mmol, 35%) as a yellow solid, mp 131-133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28-2.36 (1H, m, CH,

$NO_2CH_2C\underline{H_2}CHCO$), 2.39-2.48 (1H, m, CH, $NO_2CH_2C\underline{H_2}CHCO$), 2.73-2.82 (1H, m, CH, $NO_2CH_2CH_2C\underline{H}CO$), 2.95 (1H, dd, *J* 5.4, 13.4 Hz, CH, $NO_2CH_2CH_2CHC\underline{H_2}$), 3.17 (1H, dd, *J* 9.2, 13.2 Hz, CH, $NO_2CH_2CH_2CHC\underline{H_2}$), 3.86 (3H, s, CH, $OC\underline{H_3}$), 4.45-4.59 (2H, m, 2 x CH, $NO_2C\underline{H_2}CH_2CHCH_2$), 7.33-7.38 (3H, m, ArH), 7.74 (2H, t, *J* 8.0 Hz, ArH), 7.89 (1H, s, ArH), 7.93 (1H, s, NH), 8.08 (2H, d, *J* 8.4 Hz, 2 x CH, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.22 (CH_2), 39.01 (CH_2), 46.81 ($NO_2CH_2CH_2\underline{C}HCO$), 52.58 ($OCH_3$), 73.70 ($NO_2\underline{C}H_2CH_2CHCO$), 121.27 (CH, Ar), 124.10 (2 x CH, Ar), 124.98 (CH, Ar), 126.20 (CH, Ar), 129.47 (CH, Ar), 130.07 (2 x CH, Ar), 131.06 (C, Ar), 137.48 (C, Ar), 146.13 (C, Ar), 147.15 (C, Ar), 166.88 (C=O), 171.27 (C=O). ν_{max} (solid)/(cm^{-1}) 1684 (st), 1671 (st), 1551 (st), 1516 (st), 1447 (st), 1430 (st), 1345 (st), 1235 (st), 756 (st). MS *m/z* (**API-ES**): found 419 $(M+NH_4)^+$ (100%). HRMS *m/z* (**API-ES**): found 402.1301 $(M+H)^+$, calculated for $C_{19}H_{20}N_3O_7$ 402.1301; found 419.1570 $(M+NH_4)^+$, calculated for $C_{19}H_{23}N_4O_7$ 419.1567.

Methyl 4-[5-nitro-3-(4-nitrophenyl)pentanoylamino]benzoate **(151a).** This was prepared from corresponding amide **145f** (0.211 g, 0.647 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using the FlashMaster 3 purification station (80:20 hexanes/ethyl acetate) afforded **151a** (0.025 g, 0.062 mmol, 10%) as an off-white solid, mp 158-160 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.24-2.33 (1H, m, CH, $NO_2CH_2C\underline{H_2}CHCH_2$), 2.52-2.60 (1H, m, CH, $NO_2CH_2C\underline{H_2}CHCH_2$), 2.64-2.77 (2H, m, CH, $NO_2CH_2CH_2CHC\underline{H_2}$), 2.42-3.49 (1H, m, CH, $NO_2CH_2CH_2C\underline{H}CH_2$), 4.18 (2H, t, *J* 7.6 Hz, CH, $NO_2C\underline{H_2}CH_2CHCH_2$), 7.25 (1H, s, ArH), 7.36 (2H, d, *J* 8.2 Hz, 2 x CH, Ar), 7.42 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.90 (2H, d, *J* 8.2 H, 2 x CH, Ar), 8.14 (2H, d, *J* 8.8 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.89 (CH_2), 39.31 (CH_2), 44.04 (CH), 52.33 (OCH_3), 73.35 ($NO2\underline{C}H_2CH_2CHCH_2CO$), 119.12 (2 x CH, Ar), 124.62 (2 x CH, Ar), 128.68 (2 x CH, Ar), 131.10 (2 x CH, Ar), 141.49 (C, Ar), 147.62 (C, Ar), 149.04 (C, Ar), 166.64 (C=O), 168.05 (C=O). ν_{max} (solid)/(cm^{-1}) 3248 (md), 1755 (st), 1641 (st), 1542 (st), 1560 (st), 1321 (st), 1255 (st). MS *m/z* (**API-ES**): found 402 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 402.1301 $(M+H)^+$, calculated for $C_{19}H_{20}N_3O_7$ 402.1301; found 419.1570 $(M+NH_4)^+$, calculated for $C_{19}H_{23}N_4O_7$ 419.1567.

Methyl 4-[4-Nitro-2-(4-nitrobenzyl)butyrylamino]benzoate **(151b).** This was prepared from corresponding amide **145f** (0.211 g, 0.647 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using the FlashMaster 3 purification station (80:20 hexanes/ethyl acetate) afforded **151b** (0.051 g, 0.127 mmol, 20%) as an off-white solid, mp 150-152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.26-2.36 (1H, m, CH, $NO_2CH_2C\underline{H_2}CHCO$), 2.39-2.48 (1H, m, CH, $NO_2CH_2C\underline{H_2}CHCO$), 2.73-2.82 (1H, m, CH, $NO_2CH_2CH_2C\underline{H}CO$), 2.95 (1H, dd, *J* 5.4, 13.8 Hz, CH, $NO_2CH_2CH_2CHC\underline{H_2}$), 3.19 (1H, dd, *J* 9.4, 13.4 Hz, CH, $NO_2CH_2CH_2CHC\underline{H_2}$), 3.88 (3H, s, OCH_3), 4.44-53 (1H, m, CH, $NO_2C\underline{H_2}CH_2CHCH_2$), 4.54-4.59 (1H, m, CH, $NO_2C\underline{H_2}CH_2CHCH_2$), 7.35 (2H, d, *J* 9.0 Hz, 2

x CH, Ar), 7.48 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.86 (1H, bs, NH), 7.92 (2H, d, *J* 9.0 Hz, 2 x CH, Ar), 8.59 (2H, d, *J* 8.6 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.39 (CH_2), 38.90 (CH_2), 47.06 (CH), 52.37 (OCH_3), 73.68 ($NO_2\underline{C}H_2CH_2CHCO$), 119.41 (2 x CH, Ar), 124.17 (2 x CH, Ar), 126.53 (C, Ar), 130.04 (2 x CH, Ar), 131.04 (2 x CH, Ar), 141.29 (C, Ar), 145.94 (C, Ar), 147.23 (C, Ar), 166.72 (C=O), 171.17 (C=O). ν_{max} (solid)/(cm^{-1}) 3292 (md), 1710 (st), 1660 (st), 1552 (st), 1520 (st), 1342 (st), 1277 (st). MS *m/z* (**API-ES**): found 419 $(M+NH_4)^+$ (100%), 402 $(M+H)^+$ (40%). HRMS *m/z* (**API-ES**): found 402.13018 $(M+H)^+$, calculated for $C_{19}H_{20}N_3O_7$ 402.1302; found 419.1571 $(M+ NH_4)^+$, calculated for $C_{19}H_{23}N_4O_7$ 419.1567.

4-Nitro-3,*N*-diphenylbutyramide (144d).

A mixture of **145q** (0.089 g, 0.399 mmol), DBU (0.066 g, 0.438 mmol) in nitromethane (3 ml), was stirred in the microwave reactor at 150 °C for 15 min. After cooling to room temperature, the reaction mixture was poured into HCl (aq, 1M, 5 ml). The product was extracted with ethyl acetate (2 x 10 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure. Chromatography on silica gel performed using the FlashMaster 3 purification station (70:30 hexanes/ethyl acetate) afforded **144d** (0.055 g, 0.193 mmol, 49%) as a yellow solid, mp 123-124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.72 (1H, dd, *J* 7.2, 14.8 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 2.79 (1H, dd, *J* 7.4, 15.0 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 4.06 (1H, quint, *J* 7.2 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.70 (1H, dd, *J* 7.8, 12.6 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 4.82 (1H, dd, *J* 6.6, 12.6 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 7.09 (1H, t, *J* 7.6 Hz, ArH), 7.21-7.37 (9H, m, 8 x CH, ArH, & NH).^{13}C NMR (100 MHz, $CDCl_3$) δ 40.81 ($NO_2CH_2\underline{C}HCH_2CO$), 40.84 ($NO_2CH_2CH\underline{C}H_2CO$), 79.56 ($NO_2\underline{C}H_2CHCH_2CO$), 120.37 (2 x CH, Ar), 124.95 (CH, Ar), 127.57 (2 x CH, Ar), 128.33 (CH, Ar), 129.25 (2 x CH, Ar), 129.41 (2 x CH, Ar), 137.48 (C, Ar), 138.70 (C, Ar), 168.26 (C=O). ν_{max} (solid)/(cm^{-1}) 3351 (md), 1654 (st), 1598 (st), 1553 (st), 1524 (st), 1496 (st), 1440 (st), 1384 (st), 752 (st), 693 (st). MS *m/z* (**API-ES**): found 285 $(M+H)^+$ (100%). HRMS *m/z* (API-ES): found 285.1240 $(M+H)^+$, calculated for $C_{16}H_{17}N_2O_3$ 285.1239.

2-Methyl-3-(4-nitrophenyl)acrylic acid (157).[380,381]

Potassium carbonate (0.319 g, 1.73 mmol) was added to a solution of 4-nitro benzaldehyde (**155**) (0.285 g, 1.88 mmol) and dry piperidine (0.431 g, 5.07 mmol) in dry DCM (5 ml) under Ar at room temperature. The reaction mixture was stirred overnight at room temperature. The excess of K_2CO_3 was filtered and washed with DCM (5 ml). The filtrate was collected and the solvent removed under reduced pressure. Pyridine (2 ml) and malonic acid (0.444 g, 3.76 mmol) were added to the oil residue and the reaction mixture was stirred for 1h at 100 °C under Ar. After cooling to room temperature, HCl (aq, 1M, 10 ml) was added and the precipitate was filtered, washed with water (10 ml) and dried *under vacuum*. The acid **157**

was obtained as a yellow solid (0.205 g, 0.989 mmol, 53%) and was used in the next step without further purification.

***2-Methyl-3-(4-nitrophenyl)acryloyl chloride* (158).** This was obtained as a yellow solid (0.200 g, 0.888 mmol, 90%) from corresponding acid **157** (0.205 g, 0.990 g) in a similar manner as described for preparation of **147b**. The acid chloride was used in the next step without further purification.

***Methyl 2-[2-methyl-3-(4-nitrophenyl)acryloylamino]benzoate* (154).** This was obtained as a yellow solid (0.207 g, 0.608 mmol, 70%) from corresponding acid chloride **158** (0.200 g, 0.888 mmol) and aniline **148a** (0.149 g, 0.986 ml) in a similar manner as described for preparation of **145g**, mp 230-232 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21 (3H, d, *J* 1.2 Hz, CH_3), 3.88 (3H, s, OCH_3), 7.05-7.09 (1H, m, ArH), 7.48 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.51-7.56 (3H, m, 2 x CH, ArH, H-3), 8.01 (1H, dd, *J* 1.4, 8.3 Hz, ArH), 8.20 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 8.78 (1H, d, *J* 8.3 Hz, ArH), 11.69 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) 14.63 (CH_3), 52.74 (OCH_3), 115.51 (C, Ar), 120.79 (CH), 123.13 (CH), 123.88 (2 x CH, Ar), 130.39 (2 x CH, Ar), 131.23 (CH), 133.36 (CH), 135.08 (CH), 136.32 (C, Ar), 141.75 (C, Ar), 143.13 (C, Ar), 147.28 (C, Ar), 167.15 (C=O), 169.21 (C=O). ν_{max} (solid)/(cm^{-1}) 3625 (st), 1689 (st), 1671 (st), 1608 (st), 1589 (st), 1534 (st), 1445 (st), 1339 (st), 1258 (st), 1236 (st). MS *m/z* (**API-ES**): found 341 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 341.1124 $(M+H)^+$, calculated for $C_{18}H_{17}N_2O_5$ 341.1137; found 190.0495 $(M-C_8H_8O_2N)^+$, calculated for $C_{10}H_8NO_3$ 190.0504; found 363.0939 $(M+Na)^+$, calculated for $C_{18}H_{16}N_2O_5Na$ 363.0957.

***(E)-3-(4-Nitrophenyl)but-2-enoic acid (160) and (Z)-3-(4-Nitrophenyl)but-2-enoic acid* (161).**[382] 1.2-Dimethoxyethanetriethylphosphono acetate (1.59 g, 7.11 mmol) was added dropwise to a suspension of NaH (60% suspension in mineral oil, 0.187 g, 7.82 mmol) in anhydrous THF (10 ml) at 0 °C under Ar. After the gas evolution ceased, 4-nitroacetophenone **159** (1.068 g, 6.46 mmol) was added portionwise. The reaction mixture was stirred at room temperature under Ar overnight. The solvent was removed under reduced pressure. Ammonium chloride (aq, sat. solution, 20 ml) was added and the mixture was extracted with ethyl acetate (2 x 60 ml). The organic extracts were collected, dried over Na_2SO_4, and the solvent removed under reduced pressure. Chromatography on silica gel performed by the Flash Master 3 purification station (90:10 hexanes/ethyl acetate) afforded **160** (0.435 g, 1.85 mmol, 29%) as a white solid and **161** (0.257 g, 1.09 mmol, 17%) as a colourless oil.

160. mp 124-125 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (3H, t, *J* 7.1 Hz, $CH_2C\underline{H}_3$), 2.58 (3H, d, *J* 1.4 Hz, CH_3), 4.23 (3H, q, *J* 7.1 Hz, $C\underline{H}_2CH_3$), 6.18 (1H, d, *J* 1.4 Hz, $CH_3C\underline{H}CONH$), 7.61 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 8.23 (2H, d, *J* 9.2 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.51 ($CH_2\underline{C}H_3$), 18.15 (CH_3), 60.52 ($\underline{C}H_2CH_3$), 120.39

(CH), 124.03 (2 x CH, Ar), 127.46 (2 x CH, Ar), 148.82 (C, Ar), 152.91 (C, Ar), 166.33 (C=O). ν_{max} (solid)/(cm^{-1}) 1711 (st), 1632 (st), 1595 (st), 1512 (st), 1340 (st), 1275 (st), 1178 (st), 1041 (st), 847 (st). MS *m/z* (**API-ES**): found 236 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 236.0909 $(M+H)^+$, calculated for $C_{12}H_{14}NO_4$ 236.0923.

161. ^1H NMR (400 MHz, $CDCl_3$) δ 1.11 (3H, t, *J* 7.3 Hz, $CH_2\underline{CH_3}$), 2.19 (3H, d, *J* 1.6 Hz, CH_3), 4.23 (3H, q, *J* 7.3 Hz, $C\underline{H_2}CH_3$), 6.00 (1H, d, *J* 1.6 Hz, $CH_3C\underline{H}CONH$), 7.34 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 8.22 (2H, d, *J* 8.4 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.20 ($CH_2\underline{C}H_3$), 27.04 (CH_3), 60.37 ($\underline{C}H_2CH_3$), 119.59 (CH), 123.57 (2 x CH, Ar), 128.01 (2 x CH, Ar), 148.25 (C, Ar), 153.49 (C, Ar), 165.36 (C=O). ν_{max} (oil)/(cm^{-1}) 1719 (st), 1596 (st), 1517 (st), 1343 (st), 1233 (st), 1160 (st), 1044 (st), 853 (st). MS *m/z* (**API-ES**): found 236 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 236.0906 $(M+H)^+$, calculated for $C_{12}H_{14}NO_4$ 236.0923.

***3-(4-Nitrophenyl)-but-2-enoic acid* (162).**[382] A solution of **160** (0.396 g, 1.68 mmol) in ethanol (2 ml) was stirred in presence of KOH (aq, 1.5 M, 0.227 ml) at 100 °C for 1h. The solvent was removed under reduced pressure HCl (aq, 1M, 5 ml) was added and the white precipitate was filtered, washed with water (5 ml) and dried under vacuum. Pure **162** was obtained as a yellow solid (0.297 g, 1.43 mmol, 85%) without further purification, mp 157-159 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 2.62 (3H, d, *J* 1.6 Hz, CH_3), 6.22 (1H, d, *J* 1.6 Hz, $CH_3CC\underline{H}CONH$), 7.63 (2H, d, *J* 9.0 Hz, 2 x CH, Ar), 8.25 (2H, d, *J* 9.0 Hz, 2 x CH, Ar). ν_{max} (solid)/(cm^{-1}) 2969 (st), 1690 (st), 1622 (st), 1597 (st), 1515 (st), 1341 (st), 1279 (st), 1216 (st). MS *m/z* (**API-ES**): found 206 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 206.0449 $(M+H)^+$, calculated for $C_{10}H_8NO_4$ 206.0453.

***3-(4-Nitrophenyl)-but-2-enoic acid* (163).** This was obtained as a yellow solid (0.176 g, 0.85 mmol, 97%) from corresponding ester **161** (0.206 g, 1.36 mmol) in a similar manner as described for preparation of **162**, The crude acid was used in the next step without further purification.

***3-(4-Nitrophenyl)but-2-enoyl chloride* (164)** This was obtained as a yellow solid (0.287 g, 1.27 mmol, 94%) from corresponding acid **162** (0.282 g, 1.36 mmol) in a similar manner as described for preparation of **147b**. The acid chloride was used in the next step without further purification.

***3-(4-Nitrophenyl)but-2-enoyl chloride* (165)[34].** This was obtained as a yellow oil (0.182 g, 0.808 mmol, 98%) from corresponding acid **163** (0.166 g, 0.821 mmol) in a similar manner as described for preparation of **147b**. The acid chloride was used in the next step without further purification.

***Methyl 2-[3-(4-nitrophenyl)but-2-enoylamino]benzoate* (153).** This was obtained as a yellow solid (0.275 g, 0.808 mmol, 66%) from corresponding acid chloride **164** (0.277 g, 1.23 mmol) and aniline **148a** (0.204 g, 1.35 mmol) in a similar manner as described for preparation of **145g**, mp 229-230 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.59 (3H, d, *J* 1.2 Hz, CH_3), 3.86 (3H, s, OCH_3), 6.23 (1H, d, *J* 1.2 Hz, $CH_3CC\underline{H}CONH$), 7.03-7.07 (1H, m, ArH), 7.49-7.54 (1H, m, ArH), 7.58 (2H, d, *J* 8.8 Hz, 2 x CH, ArH), 7.99 (1H, dd, *J* 1.6, 8.4 Hz, ArH), 8.18 (2H, d, *J* 8.8 Hz, 2 x CH, ArH), 8.76 (1H, d, *J* 8.4 Hz, ArH), 11.27 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.09 (CH_3), 52.64 (OCH_3), 115.09 (C, Ar), 120.53 (CH, Ar), 122.96 (CH, Ar), 123.85 (CH, Ar), 124.02 (2 x CH, Ar), 127.48 (2 x CH, Ar), 131.71 (CH, Ar), 134.98 (CH, Ar), 141.86 (C, Ar), 148.00 (C, Ar), 149.19 (C, Ar), 150.81 (C, Ar), 164.68 (C=O), 169.13 (C=O). ν_{max} (solid)/(cm^{-1}) 3259 (st), 1671 (st), 1600 (st), 1513 (st), 1445 (st), 1433 (st), 1342 (st), 1257 (st). MS *m/z* (**API-ES**): found 341 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found found 341.1135 $(M+H)^+$, calculated for $C_{18}H_{17}N_2O_5$ 341.1137.

***Methyl 2-[3-(4-nitrophenyl)but-2-enoylaino]benzoate* (166).** This was obtained as a yellow solid (0.077 g, 0.226 mmol, 26%) from corresponding acid chloride **165** (0.182 g, 0.879 mmol) and aniline **148a** (0.146 mmol, 0.966 mmol) in a similar manner as described for preparation of **145g**. Chromatography on silica gel performed by the Flash Master 3 purification station (60:40 hexanes/ethyl acetate) afforded **166** (0.077 g, 0.226 mmol, 26%) as a yellow solid, mp 225-227 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23 (3H, d, *J* 1.2 Hz, CH_3), 3.89 (3H, s, O CH_3), 6.16 (1H, d, *J* 1.2 Hz, $CH_3CC\underline{H}CONH$), 7.02-7.07 (1H, m, ArH), 7.42-7.47 (3H, m, ArH), 7.98 (1H, dd, *J* 1.6, 8.2 Hz, ArH), 8.02 (2H, d, *J* 8.9 Hz, 2 x CH, ArH), 8.57 (1H, d, *J* 8.2 Hz, ArH), 11.08 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.75 (CH_3), 52.52 (OCH_3), 115.04 (C, Ar), 120.64 (CH), 122.90 (CH), 123.55 (CH), 123.66 (2 x CH, Ar), 128.32 (2 x CH, Ar), 131.04 (CH), 134.85 (CH), 141.50 (C, Ar), 147.40 (C, Ar), 148.05 (C, Ar), 149.87 (C, Ar), 163.86 (C=O), 168.89 (C=O). ν_{max} (solid)/(cm^{-1}) 3312 (st), 3300 (st), 1700 (st), 1684 (st), 1587 (st), 1508 (st), 1426 (st), 1343 (st), 1259 (st), 1238 (st), 1177 (st), 1161 (st), 1086 (st). MS *m/z* (**API-ES**): found 341 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 341.1123 $(M+H)^+$, calculated for $C_{18}H_{17}N_2O_5$ 341.1137, found 190.0494 $(M-C_8H_8O_2N)^+$, calculated for $C_{10}H_8NO_3$ 190.0504; found 703.2007 $(2M+Na)^+$, calculated for $C_{36}H_{32}N_4O_{10}Na$ 703.2016.

***Methyl 4-(1-nitromethyl-2-carboxylphenylcarbamoyl-ethyl)-benzoate* (144o).** This was prepared from corresponding amide **145a** (0.081 g, 0.238 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel using the FlashMaster 3 purification station (80:20 hexanes/ethyl acetate) afforded **144o** (0.046 g, 0.115 mmol, 48%) as a yellow solid, mp 189-190 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.86 (1H, dd, *J* 7.4, 15.6 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 2.93 (1H, dd, *J* 7.4, 15.6 Hz, CH, $NO_2CH_2CHC\underline{H_2}CO$), 3.89 (1H, s, OCH_3), 3.91 (1H, s, OCH_3), 4.20 (1H, quint, *J* 7.2 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.74

(1H, dd, *J* 12.8, 8.4 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 4.88 (1H, dd, *J* 12.8, 6.0 Hz, CH, $NO_2C\underline{H_2}CHCH_2CO$), 7.07-7.11 (1H, m, ArH), 7.37 (1H, d, *J* 8.4 Hz, ArH), 7.50-7.54 (1H, m, ArH), 7.93 (3H, m, ArH), 8.59 (1H, dd, *J* 0.8, 8.4 Hz, ArH), 11.15 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.50 , ($NO_2CH_2CH\underline{C}H_2CO$), 41.34 , ($NO_2CH_2\underline{C}HCH_2CO$), 52.39 , ($OCH_3$), 52.66 , ($OCH_3$), 79.18 , ($NO_2\mathbf{C}H_2CHCH_2CO$), 115.17 (C, Ar), 120.60 (CH, Ar), 123.18 (CH, Ar), 127.78 (2 x CH, Ar), 130.10 (C, Ar), 130.60 (2 x CH, Ar), 131.07 (CH, Ar), 134.97 (CH, Ar), 141.11 (C, Ar), 143.87 (C, Ar), 166.74 (C=O), 168.24 (C=O), 168.98 (C=O). ν_{max} (oil)/(cm^{-1}) 3259 (st), 2952 (st), 2922 (st), 1723 (st), 1702 (st), 1681 (st), 1601 (st), 1588 (st), 1551 (st), 1528 (st), 1432 (st), 1258 (st). MS *m/z* (**API-ES**): found 401 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 401.1341 $(M+H)^+$, calculated for $C_{20}H_{21}N_2O_7$ 401.1349.

Methyl 4-(1-methyl-2-carboxyphenylcarbamoyl-vinyl)-benzoate **(175)**. This was prepared from **144o** (0.011 g, 0.0275 mmol) in a similar manner as described for preparation of **144d**. Chromatography on silica gel performed by the FlashMaster 3 purification station (80:20 hexanes/ethyl acetate) afforded **165** (0.004 g, 0.011 mmol, 41%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.58 (3H, d, *J* 1.2 Hz, CH_3), 3.88 (3H, s, OCH_3), 3.89 (1H, s, OCH_3), 6.21 (1H, d, *J* 1.2 Hz, $CH_3CC\underline{H}CONH$), 7.01-7.05 (1H, m, ArH), 7.48-7.53 (3H, m, ArH), 7.98 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 8.77 (1H, dd, *J* 0.8, 8.4 Hz, ArH), 11.20 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.99 (CH_3), 52.88 (OCH_3), 116.10 (C, Ar), 121.34 (CH, Ar), 123.01 (CH, Ar), 123.70 (CH, Ar), 124.14 (2 x CH, Ar), 127.65 (2 x CH, Ar), 131.67 (CH, Ar), 135.01 (CH, Ar), 141.93 (C, Ar), 147.90 (C, Ar), 149.22 (C, Ar), 150.76 (C, Ar), 163.78 (C=O), 168.11 (C=O), 169.18. ν_{max} (oil)/(cm^{-1}) 3279 (st), 1755 (st), 1677 (st), 1688 (st), 1645 (st), 1590 (st), 1531 (st), 1435 (st), 1474 (st), 1236 (st). MS *m/z* (**API-ES**): found 354 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 354.1339 $(M+H)^+$, calculated for calculated for $C_{20}H_{20}NO_5$ 354.1341

Methyl 4-[1-methyl-2-(2-nitrophenylcarbamoyl)vinyl]benzoate **(176)**. This was prepared from corresponding amide **145s** (0.085 g, 0.260 mmol) in a similar manner as described for preparation of **144l**. Chromatography on silica gel performed using the FlashMaster 3 purification station (80:20 hexanes/ethyl acetate) afforded **145s** (0.046 g, 0.132 mmol, 51%) as a off white solid, mp 227-229 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.58 (3H, s, CH_3), 3.87 (3H, s, OCH_3), 6.20 (1H, s, $CH_3CC\underline{H}CONH$), 7.12 (1H, t, *J* 7.4 Hz, ArH), 7.49 (2H, d, *J* 7.2 Hz, 2 x CH, Ar), 7.60 (1H, t, *J* 7.4 Hz), 7.99 (2H, d, *J* 7.2 Hz, 2 x CH, Ar), 8.16 (1H, d, *J* 7.4 Hz), 8.89 (1H, d, *J* 7.4 Hz), 10.45 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.35, (CH_3), 52.49 , (OCH_3), 121.47 (CH), 122.30 (CH), 123.41 (CH), 126.07 (CH), 126.58 (2 x CH, Ar), 130.11 (2 x CH, Ar), 130.78 (C, Ar), 135.45 (C, Ar), 136.16 (C, Ar), 136.52 (CH), 147.77 (C, Ar), 154.55 (C, Ar), 164.95 (C=O), 166.76 (C=O). ν_{max} (solid)/(cm^{-1}) 3359 (st), 2958 (st), 2924 (st), 1714 (st), 1691 (st), 1604 (st), 1584 (st), 1496 (st), 1427 (st), 1335 (st),

1278 (st), 1265 (st), 1153 (st), 1144 (st). MS *m/z* (**API-ES**): found 341 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 341.1120 $(M+H)^+$, calculated for $C_{18}H_{17}N_2O_5$ 341.1137; found 203.0696 $(M-C_6H_5O_2N_2)^+$, calculated for $C_{12}H_{11}O_3$ 203.0708; found 703.2007 $(2M+Na)^+$, calculated for $C_{36}H_{32}N_4O_{10}Na$ 303.2016.

***Ethyl 4-(4-amino-3-phenylbutyrylamino)benzoate* (143a)**[385]

To a stirred solution of **144a** (0.749 g, 2.10 mmol) and $NiCl_2 \cdot 6H_2O$ (1.99 g, 8.40 mmol) in methanol (10 ml) $NaBH_4$ (0.719 g, 18.93 mmol) was added portionwise over 20 min at 0 °C. After stirring for 15 at room temperature, the solvent was removed under reduced pressure. Water (20 ml) and ethyl acetate (40 ml) were added to the solid residue. The resulting mixture was filtered through a celite bed which was washed with ethyl acetate (20 ml). After collecting the filtrate, the organic phase was separated, dried over Na_2SO_4 and the solvent removed under reduced pressure to afford **143a** as an off white solid (0.596 g, 1.83 mmol, 87%). The crude compound used in the next step without further purification.

***Ethyl 4-(4-amino-3-naphthalen-2-yl-butyrylamino)benzoate* (143b)**. This was obtained as a yellow solid (0.819 g, 2.178 mmol, 89%) from **144b** (0.995 g, 2.450 mmol) in a similar manner as described for preparation of **143a**. The crude compound used in the next step without further purification.

Ethyl 4-[3-phenyl-4-(toluene-4-sulfonylamino)butyrylamino]benzoate (142a)

Potassium carbonate (0.325 g, 2.35 mmol) was added to a solution of **143a** (0.128 g, 0.392 mmol) in 1,4-dioxane/H_2O 1:1 (5 mL) and followed by tosyl chloride (0.074 g, 0.392 mmol) at room temperature. After stirring for 2 h at room temperature, the resulting mixture was evaporated *in vacuo* to dryness. Water (10 ml) was added and the formed white solid was separated by filtration and dried *in vacuo*. Pure **142a** was obtained as an off-white solid (0.122 g, 0.254 mmol, 65%) without further purification, mp 135-137 °C. ^{1}H NMR (400 MHz, CD_3OD) 1.36 (3H, t, *J* 7.2 Hz, $CH_2C\underline{H}_3$), 2.39 (3H, s, CH_3), 2.63 (1H, dd, *J* 8.6, 14.6 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.85 (1H, dd, *J* 6.2, 15.0 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.05 (1H, dd, *J* 7.4, 13.0 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.12 (1H, dd, *J* 6.8, 11.8 Hz, CH, $C\underline{H}_2CHCH_2CO$), 4.32 (2H, q, *J* 7.2 Hz, $CH_2C\underline{H}_3$), 7.17-7.19 (3H, m, ArH), 7.23-7.27 (2H, m, ArH), 7.31 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.54 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.65 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.90 (2H, d, *J* 8.4 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) 14.57 ($CH_2\underline{C}H_3$), 21.73 (CH_3), 40.65 ($CH_2CH\underline{C}H_2CO$), 42.04 ($CH_2\underline{C}HCH_2CO$), 47.77 ($\underline{C}H_2CHCH_2CO$), 61.07 ($\underline{C}H_2CH_3$), 119.15 (CH, Ar), 127.14 (2 x CH, Ar), 127.75 (2 x CH, Ar), 127.77 (2 x CH, Ar), 129.29 (2 x CH, Ar), 130.07 (2 x CH, Ar), 130.94 (2 x CH, Ar), 136.79 (C, Ar), 140.74 (C, Ar), 142.12 (C, Ar), 143.97 (C, Ar), 166.36 (C=O), 170.24 (C=O). ν_{max} (solid)/(cm^{-1}) 3324 (st), 3191 (md), 1707 (st), 1675 (st), 1597 (st), 1534 (st), 1270 (st), 1157

(st), 1105 (st). MS *m/z* (**API-ES**): found 481 $(M+H)^+$ (100 %). HRMS *m/z* (**API-ES**): found 481.18010 $(M+H)^+$, calculated for $C_{26}H_{29}N_2O_5S$ 481.1797.

Ethyl 4-[4-(4-phenoxybenzenesulfonylamino)-3-phenylbutyrylamino]benzoate **(142b)**. This was obtained as a off white solid (0.182 g, 0.326 mmol, 77%) from **133a** (0.137 g, 0.420 mmol) and 4-phenoxybenzenesulfonyl chloride (0.112 g, 0.420 mmol) in a similar manner as described for preparation of **142a**, mp 147-149 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 1.36 (3H, t, *J* 7.2 Hz, $CH_2C\underline{H}_3$), 2.63 (1H, dd, *J* 8.4, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.84 (1H, dd, *J* 7.4, 13.2 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.09 (1H, dd, *J* 7.2, 12.8 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.16 (1H, dd, *J* 7.2, 12.4 Hz, CH, $C\underline{H}_2CHCH_2CO$), 4.31 (2H, q, *J* 7.2 Hz, $CH_2C\underline{H}_3$), 7.00 (2H, d, *J* 8.0 Hz, 2 x CH, Ar), 7.06 (2H, dd, *J* 0.8, 8.8 Hz, Ar), 7.18-7.26 (6H, m), 7.39-7.43 (2H, m, ArH), 7.54 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.73 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.89 (2H, d, *J* 8.0 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) 14.57 ($CH_2\underline{C}H_3$), 40.73 ($CH_2CH\underline{C}H_2CO$), 42.06 ($CH_2\underline{C}HCH_2CO$), 47.80 ($\underline{C}H_2CHCH_2CO$), 61.07 ($\underline{C}H_2CH_3$), 117.92 (2 x CH, Ar), 119.15 (CH, Ar), 120.52 (2 x CH, Ar), 125.25 (CH, Ar), 127.75 (CH, Ar), 127.80 (CH, Ar), 129.32 (2 x CH, Ar), 129.35 (2 x CH, Ar), 130.41 (2 x CH, Ar), 130.95 (2 x CH, Ar), 133.27 (C, Ar), 140.74 (C, Ar), 142.08 (C, Ar), 155.23 (C, Ar), 161.95 (C, Ar), 166.34 (C=O), 170.20 (C=O). ν_{max} (solid)/(cm^{-1}) 3322 (st), 1702 (st), 1596 (st), 1532 (st), 1487 (st), 1407 (st), 1274 (md), 1243 (st), 1151 (st), 1104 (md), 695 (st). MS *m/z* (**API-ES**): found 559 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 559.1906 $(M+H)^+$, calculated for $C_{31}H_{31}N_2O_6S$ 559.1903.

Ethyl 4-[3-naphthalen-2-yl-4-(toluene-4-sulfonylamino)butyrylamino]benzoate **(142c)**. This was obtained as an off white solid (0.058 g, 0.109 mmol, 33%) from **143b** (0.138 g, 0.375 mmol) in a similar manner as described for preparation of **142a**, mp 122-124°C. ^{1}H NMR (400 MHz, CD_3OD) δ 1.34 (3H, t, *J* 7.2 Hz, $C\underline{H}_2CH_3$), 2.34 (3H, s, CH_3), 2.73 (1H, dd, *J* 8.2, 14.6 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.92 (1H, dd, *J* 6.4, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.19 (1H, dd, *J* 7.8, 13.4 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.17-3.28 (1H, m, CH, $C\underline{H}_2CHCH_2CO$), 3.47-3.52 (1H, m, CH, $CH_2C\underline{H}CH_2CO$), 4.30 (2H, q, *J* 7.2 Hz, $C\underline{H}_2CH_3$), 7.19 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.32 (1H, dd, *J* 1.4, 8.6 Hz, ArH), 7.40-7.45 (2H, m, ArH), 7.51 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.59 (2H, d, *J* 8.6 Hz, 2 X CH, Ar), 7.60 (1H, s, ArH), 7.73-7.79 (3H, m, ArH), 7.90 (2H, d, *J* 8.6 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, CD_3OD) δ 14.55 ($CH_2\underline{C}H_3$), 21.54 (CH_3), 40.45 ($CH_2CH\underline{C}H_2CO$), 42.56 ($CH_2\underline{C}HCH_2CO$), 48.31 ($\underline{C}H_2CHCH_2CO$), 59.98 ($CH_2\underline{C}H_3$), 118.95 (2 x CH, Ar), 125.63 (C, Ar), 127.22 (2 x CH, Ar), 127.51 (CH, Ar), 128.40 (2 x CH, Ar), 129.07 (2 x CH, Ar), 130.33 (2 x CH, Ar), 130.98 (2 x CH, Ar), 137.09 (C, Ar), 142.55 (C, Ar), 143.23 (C, Ar), 143.70 (C, Ar), 167.96 (C=O), 170.14 (C=O). ν_{max} (solid)/(cm^{-1}) 2958 (md), 1706 (st), 1596 (st), 1531 (st), 1273 (st), 1171 (st), 1152 (st), 1105 (st), 1020 (st). MS *m/z* (**API-ES**): found 531 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 531.19624 $(M+H)^+$, calculated for $C_{30}H_{31}N_2O_5S$ 531.1954.

***Ethyl 4-[3-naphthalen-2-yl-4-(4-phenoxybenzenesulfonylamino)butyrylamino]benzoate* (142d).** This was obtained as a white solid in (0.100 g, 0.164 mmol, 73%) from **143b** in a similar manner as described for preparation of **142a**, mp 141-143 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 1.34 (3H, t, *J* 7.2 Hz, $CH_2C\underline{H}_3$), 2.75 (1H, dd, *J* 8.4, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.92 (1H, dd, *J* 6.6, 15.0 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.25-3.29 (2H, m, 2 x CH, $C\underline{H}_2CHCH_2CO$), 3.34-3.53 (1H, m, CH, $CH_2C\underline{H}CH_2CO$) 4.30 (2H, q, *J* 7.2 Hz, $C\underline{H}_2CH_3$), 6.61 (2H, d, *J* 9.2 H, 2 x CH, Ar), 7.05 (2H, dd, *J* 1.3, 8.7 Hz, ArH), 7.20-7.23 (1H, m, ArH, ArH), 7.34 (1H, dd, *J* 1.3, 8.7 Hz, ArH), 7.40-7.45 (4H, m, ArH), 7.54 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 6.64 (1H, bs, ArH), 7.67 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.75-7.90 (3H, m, ArH), 7.87 (2H, d, *J* 9.2 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) 14.55 ($CH_2\underline{C}H_3$), 40.68 ($CH_2C\underline{H}CH_2CO$), 42.12 ($CH_2\underline{C}HCH_2CO$), 47.84 ($\underline{C}H_2CHCH_2CO$), 61.05 ($CH_2\underline{C}H_3$), 117.89 (2 x CH, Ar), 119.19 (CH, Ar), 120.51 (2 x CH, Ar), 125.22 (CH, Ar), 125.67 (CH, Ar), 126.21 (CH, Ar), 126.35 (CH, Ar), 126.53 (C, Ar), 126.73 (CH, Ar), 127.85 (CH, Ar), 127.96 (CH, Ar), 129.09 (2 x CH, Ar), 129.83 (2 x CH, Ar), 130.39 (2 x CH, Ar), 130.90 (2 x CH, Ar), 132.85 (C, Ar), 133.27 (C, Ar), 133.61 (C, Ar), 138.16 (C, Ar), 142.13 (C, Ar), 155.24 (C, Ar), 161.90 (C, Ar), 166.34 (C=O), 170.26 (C=O). ν_{max} (solid)/(cm^{-1}) 1707 (st), 1596 (st), 1531 (st), 1486 (st), 1275 (st), 1243 (st), 1151 (st), 1098 (st). MS *m/z* (**API-ES**): found 609 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 609.2058 $(M+H)^+$, calculated for $C_{35}H_{33}N_2O_6S$ 609.2059.

4-[3-Phenyl-4-(toluene-4-sulfonylamino)butyrylamino]benzoic acid (178a)

A solution of **142a** (0.106 g, 0.220 mmol) in methanol (3 ml) and THF (3 ml) was stirred in presence of NaOH (aq, 1M, 1 ml) at room temperature overnight. The solvent was removed under reduced pressure. HCl (aq, 1M, 1.5 ml) was added and the precipitate was filtered, washed with water (5 ml) and dried *under vacuum*. Pure acid **178a** was obtained as a white solid (0.085 g, 0.188 mmol, 85%) without further purification, mp 225-227 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 2.39 (3H, s, CH_3), 2.62 (1H, dd, *J* 8.4, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.35 (1H, dd, *J* 6.6, 14.6 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.05 (1H, dd, *J* 8.0, 13.0 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.12 (1H, dd, *J* 7.2, 12.8 Hz, CH, $C\underline{H}_2CHCH_2CO$), 7.16-7.19 (3H, m, ArH), 7.24-7.27 (2H, m, ArH), 7.30 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.53 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.65 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.91 (2H, d, *J* 9.2 Hz, 2 x CH, A r). ^{13}C NMR (100 MHz, DMSO-d_3) δ 21.61 (CH_3), 40.65 ($CH_2C\underline{H}CH_2CO$), 42.32 ($CH_2\underline{C}HCH_2CO$), 48.33 ($\underline{C}H_2CHCH_2CO$), 118.95 (2 x CH, Ar), 125.61 (C, Ar), 127.18 (2 x CH, Ar), 127.26 (CH, Ar), 128.38 (2 x CH, Ar), 128.96 (2 x CH, Ar), 130.02 (2 x CH, Ar), 130.98 (2 x CH, Ar), 138.09 (C, Ar), 142.47 (C, Ar), 143.22 (C, Ar), 143.72 (C, Ar), 167.55 (C=O), 170.64 (C=O). ν_{max} (solid)/(cm^{-1}) 3316 (md), 3277 (md), 1668 (st), 1595 (st), 1531 (st), 1408 (st), 1317 (st), 1254 (st), 1152 (st). MS *m/z* (**API-ES**): found 451 $(M-H)^-$ (100%), HRMS *m/z* (**API-ES**): found 451.1334 $(M-H)^-$, calculated for $C_{24}H_{23}N_2O_5S$ 451.1328.

4-[4-(4-Phenoxybenzenesulfonylamino)-3-phenylbutyrylamino]benzoic acid **(178b)**. This was obtained as a white solid (0.120 g, 0.226 mmol, 83%) from **142b** (0.152, 0.272 mmol) in a similar manner as described for preparation of **178a**, mp 187-189 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 2.63 (1H, dd, *J* 8.8, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.84 (1H, dd, *J* 6.6, 14.6 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.09 (1H, dd, *J* 7.8, 13.0 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.16 (1H, dd, *J* 6.8, 12.8 Hz, CH, $C\underline{H}_2CHCH_2CO$), 7.00 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.06 (2H, dd, *J* 1.2, 8.4 Hz, ArH), 7.16-7.28 (6H, m, ArH), 7.39-743 (2H, m, ArH), 7.53 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.73 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.90 (2H, d, *J* 8.8 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, CD_3OD) 40.86 ($CH_2\underline{C}HCH_2CO$), 42.63 ($CH_2CH\underline{C}H_2CO$), 47.85 ($\underline{C}H_2CHCH_2CO$), 117.47 (2 x CH, Ar), 119.00 (2 x CH, Ar), 120.09 (2 x CH, Ar), 124.77 (CH, Ar), 125.74 (C, Ar), 126.70 (CH, Ar), 127.66 (2 x CH, Ar), 128.46 (2 x CH, Ar), 129.12 (2 x CH, Ar), 130.10 (2 x CH, Ar), 130.49 (2 x CH, Ar), 134.36 (C, Ar), 141.39 (C, Ar), 142.91 (C, Ar), 155.62 (C, Ar), 161.59 (C, Ar), 168.23 (C=O), 171.43 (C=O). ν_{max} (solid)/(cm^{-1}) 3060 (st), 1681 (st), 1596 (st), 1585 (st), 1487 (st), 1318 (st), 1294 (st), 1251 (st), 1151 (st). MS *m/z* (**API-ES**): found 529 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 529.1433 $(M-H)^-$, calculated for $C_{29}H_{25}N_2O_6S$ 529.1433.

4-[3-Naphthalen-2-yl-4-(4-phenoxy-benzenesulfonylamino)-butyrylamino]-benzoic acid **(178d)**. This was obtained as a white solid (0.052 g, 0.090mmol, 70%) from **142d** (0.079 g, 0.129 mmol) in a similar manner as described for preparation of **178a**, mp 183-184 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 2.65 (1H, dd, *J* 8.4, 14.8 H, $CH,CH_2CHC\underline{H}_2CO$), 2.82 (1H, dd, *J* 6.8, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.11-3.20 (2H, m, CH, $C\underline{H}_2CHCH_2CO$), 3.40-3.44 (1H, m, CH, $CH_2C\underline{H}CH_2CO$), 6.81 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 6.94-6.96 (2H, m, ArH), 7.10-7.14 (1H, m, ArH), 7.25 (1H, dd, *J* 1.6, 8.8 Hz, ArH), 7.29-7.35 (4H, m, ArH), 7.41 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.54 (1H, bs, ArH), 7.58 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.65-7.68 (3H, m, ArH), 7.78 (2H, d, *J* 8.4 Hz, 2 x CH, Ar) . ^{13}C NMR (100 MHz, CD_3OD) 40.82 ($CH_2CH\underline{C}H_2CO$), 42.70 ($CH_2\underline{C}HCH_2CO$), 47.90 ($\underline{C}H_2CHCH_2CO$), 117.34 (2 x CH, Ar), 119.00 (2 x CH, Ar), 120.115 (2 x CH, Ar), 124.76 (CH, Ar), 125.53 (CH, Ar), 125.66 (CH, Ar), 125.91 (CH, Ar), 126.54 (CH, Ar), 127.39 (2 x CH, Ar), 127.53 (CH, Ar), 128.12 (CH, Ar), 129.06 (2 x CH, Ar), 130.09 (2 x CH, Ar), 130.51 (CH, Ar), 132.92 (C, Ar), 133.74 (C, Ar), 134.38 (C, Ar), 138.80 (C, Ar), 142.80 (C, Ar), 155.55 (C, Ar), 161.50 (C=O), 171.36 (C=O). ν_{max} (solid)/(cm^{-1}) 3061 (st), 1683 (st), 1599 (st), 1584 (st), 1514 (st), 1486 (st), 1304 (st), 1240 (st), 1147 (st). MS *m/z* (**API-ES**): found 579 $(M-H)^-$ (100%), HRMS *m/z* (**API-ES**): found 579.1586 $(M-H)^-$, calculated for $C_{33}H_{27}N_2O_6S$ 579.1590.

4-[3-Naphthalen-2-yl-4-(toluene-4-sulfonylamino)-butyrylamino]-benzoic acid **(178c)**. This was obtained as a white solid (0.021 g, 0.042 mmol, 65%) from **142c** (0.034 g, 0.064

mmol) in a similar manner as described for preparation of **142a**, mp 169-171 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 2.34 (3H, s, CH_3), 2.73 (1H, dd, *J* 8.2, 14.6 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.91 (1H, dd, *J* 6.4, 14.8 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.20 (1H, dd, *J* 7.6, 12.8 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.25-3.29 (1H, m, CH, $C\underline{H}_2CHCH_2CO$), 3.47-3.53 (1H, m, CH, $CH_2C\underline{H}CH_2CO$), 7.19 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.32 (1H, dd, *J* 1.6, 8.8 Hz, ArH), 7.39-7.45 (2H, m, ArH), 7.51 (2H, d, *J* 9.0 Hz, 2 x CH, Ar), 7.59 (2H, d, *J* 9.0 Hz, 2 x CH, ArH), 7.60 (1H, s, ArH), 7.73-7.79 (3H, m, ArH), 7.88 (2H, d, *J* 8.6 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.60 (CH_3), 40.88 ($CH_2CH\underline{C}H_2CO$), 42.52 ($CH_2\underline{C}HCH_2CO$), 48.31 ($\underline{C}H_2CHCH_2CO$), 118.92 (2 x CH, Ar), 125.73 (C, Ar), 126.20 (CH, Ar), 126.65 (CH, Ar), 126.88 (CH, Ar), 126.95 (CH, Ar), 127.13 (2 x CH, Ar), 128.09 (CH, Ar), 128.20 (CH, Ar), 128.41 (CH, Ar), 130.18 (2 x CH, Ar), 130.95 (2 x CH, Ar), 132.73 (C, Ar), 133.62 (C, Ar), 138.16 (C, Ar), 140.03 (C, Ar), 143.15 (C, Ar), 143.67 (C, Ar), 167.57 (C=O), 170.61 (C=O). ν_{max} (solid)/(cm^{-1}) 1680 (st), 1595 (st), 1530 (st), 1408 (st), 1305 (st), 1252 (st), 1152 (st). MS *m/z* (**API-ES**): found 501 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 501.14831 $(M-H)^-$, calculated for $C_{28}H_{25}N_2O_5S$ 501.1484.

Methyl 2-Benzyloxy-4-(3-naphthalen-2-yl-4-nitrobutyrylamino)benzoate **(180)**. Benzyl bromide (0.236 g, 1.38 mmol) and K_2CO_3 (0.190 g, 1.38 mmol) were added to a stirred solution of **145c** (0.400 g, 1.15 mmol) in anhydrous DMF (7 ml) under Ar. The reaction mixture was stirred at room temperature overnight and the mixture was poured in water (15 ml). The product was extracted with ethyl acetate (2 x 15 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure. The resulting crude material was dissolved in nitromethane (7 ml) and heated in the microwave reactor at 100 °C for 15 min in presence of DBU (0.192 g, 1.27 mmol). After cooling to room temperature, the reaction mixture was poured into HCl (aq, 1M, 10 ml). The product was extracted with ethyl acetate (2 x 15 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure. Chromatography on silica gel (60:40 hexanes/ethyl acetate, R_f 0.45) afforded **180** (0.413 g, 0.83 mmol, 72%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.88 (1H, dd, *J* 6.8, 15.6 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 2.96 (1H, dd, *J* 7.2, 15.2 Hz, CH, $NO_2CH_2CHC\underline{H}_2CO$), 3.86 (3H, s, CH, OCH_3), 4.26 (1H, quint, *J* 7.1 Hz, CH, $NO_2CH_2C\underline{H}CH_2CO$), 4.83 (1H, dd, *J* 7.4, 12.6 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 4.93 (1H, dd, *J* 6.8, 12.4 Hz, CH, $NO_2C\underline{H}_2CHCH_2CO$), 5.10 (2H, s, CH_2), 6.71 (1H, dd, *J* 2.0, 8.8 Hz, H-6'), 7.13 (1H, bs, ArH), 7.28-7.32 (1H, m, ArH), 7.36-7.39 (3H, m, ArH), 7.47-7.52 (5H, m, ArH), 7.71 (1H, bs, NH), 7.77-7.86 (4H, m, ArH). ^{13}C NMR (400 MHz, $CDCl_3$) δ 40.68 ($NO_2CH_2\underline{C}HCH_2CO$), 40.81 ($NO_2CH_2CH\underline{C}H_2CO$), 52.15 ($OCH_3$), 70.70 ($NO_2\underline{C}H_2CHCH_2CO$), 79.44 ($CH_2$), 101.39 (CH, Ar), 111.15 (CH, Ar), 115.91 (CH, Ar), 124.94 (CH, Ar), 126.67 (CH, Ar), 126.79, 126.89 (CH, Ar), 127.22 (2 x CH, Ar), 127.93 (2 x CH, Ar), 128.05 (CH, Ar), 128.79 (CH, Ar), 128.72 (CH, Ar), 129.39 (CH, Ar), 133.11 (C, Ar), 133.60 (C, Ar), 135.89 (C, Ar), 136.54 (C, Ar), 142.68 (C, Ar), 159.61 (C, Ar), 166.42 (C=O), 168.57 (C=O). ν_{max} (solid)/(cm^{-1}) 1688 (st), 1650

(st), 1597, 1532 (st), 1513 (st), 1182 (st), 1132 (st). MS *m/z* (**API-ES**): found 409 $(M+H)^+$ (100%). HRMS *m*. MS *m/z* (**API-ES**): found 499 $(M+H)^+$ (100%). HRMS *m/z* (API-ES, pos): found 499.1862 $(M+H)^+$, calculated for $C_{29}H_{27}N_2O_6$ 499.1869.

***Methyl 4-(4-amino-3-naphthalen-2-ylbutyrylamino)-2-benzyloxybenzoate* (143c).** This was obtained as a white solid (0.050 g, 0.106 mmol, 13%) from **180** (0.386 g, 0.795 mmol) a similar manner as described for preparation of **143a**. The crude compound was taken to the next step without further purification.

***Methyl 2-benzyloxy-4-[3-naphthalen-2-yl-4-(4-phenoxybenzenesulfonylamino)butyrylamino]-benzoate* (142e)**. This was obtained as an off white solid (0.023 g, 0.032 mmol, 36%) from **143c** (0.043 g, 0.091 mmol) in a similar manner as described for preparation of **142a.** The crude was used in the next step without further purification.

***Methyl 2-hydroxy-4-[3-naphthalen-2-yl-4-(4-phenoxybenzenesulfonylamino)butyrylamino]-benzoate* (181)**. A solution of **142e** (0.023 g, 0.032 mmol) in methanol (1 ml) was stirred in presence of ammonium formate (0.089 g) and 10% Pd/C (0.023 g), under H_2, at room temperature for 2 days. The solution was then filtered through a celite bed and the solvent removed under reduced pressure to afford **141** (0.014 g, 0.0230 mmol, 70%) as yellow solid, which was used in the next step without further purification.

***2-Hydroxy-4-[3-naphthalen-2-yl-4-(4-phenoxybenzenesulfonylamino)butyrylamino]benzoic acid* (178e)**. This was obtained as an off-white solid (0.0063 g, 0.011 mmol, 50%) from **181** (0.013 g, 0.021 mmol) in a similar manner as described for preparation of **142a**, mp 153-155 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 2.63 (1H, dd, *J* 14.6, 8.2 Hz, CH, $CH_2CHC\underline{H}_2CO$), 2.86 (1H, dd, *J* 15.0, 6.6 Hz, CH, $CH_2CHC\underline{H}_2CO$), 3.13 (1H, dd, *J* 13.2, 7.6 Hz, CH, $C\underline{H}_2CHCH_2CO$), 3.37-3.48 (1H, m, CH, $CH_2C\underline{H}CH_2CO$), 6.77-6.83 (3H, m, ArH), 6.94-6.96 (2H, m, ArH), 7.08-7.14 (2H, m, ArH), 7.24 (1H, dd, *J* 1.6, 8.4 Hz, ArH), 7.29-7.35 (4H, m, ArH), 7.53 (1H, bs, ArH), 7.57-7.60 (1H, m, ArH), 7.66-7.69 (3H, m, ArH), 9.80 (1H, bs, ArH). ^{13}C NMR (400 MHz, CD_3OD) δ 40.59 ($CH_2CH\underline{C}H_2CO$), 42.67 ($CH_2\underline{C}HCH_2CO$, 47.72 ($\underline{C}H_2CHCH_2CO$), 106.81 (CH, Ar), 110.12 (CH, Ar), 117.35 (2 x CH, Ar), 120.12 (2 x CH, Ar), 124.75 (CH, Ar), 125.52 (CH, Ar), 125.67 (CH, Ar), 125.90 (CH, Ar), 126.55 (CH, Ar), 127.39 (CH, Ar), 127.55 (CH, Ar), 128.13 (CH, Ar), 129.06 (2 x CH, Ar), 130.09 (2 x CH, Ar), 131.03 (CH, Ar), 132.90 (C, Ar), 133.74 (C, Ar), 134.34 (C, Ar), 138.82 (C, Ar), 143.81 (C, Ar), 155.54 (C, Ar), 161.50 (C, Ar), 162.34 (C=O), 171.32 (C=O). ν_{max} (solid)/(cm^{-1}) 3253 (st), 1641

(st), 1632 (st), 1565 (md), 1524 (st), 1291 (st), 1165 (st). MS *m/z* (**API-ES**): found 595 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 595.1537 $(M-H)^-$, calculated for $C_{33}H_{27}N_2O_7S$ 595.1539.

***4-tert-Butoxycarbonylaminobenzoic acid ethyl ester* (183)**[422]

t-B(12.68 g, 58.1 mmol) was added to a solution of ethyl-4amino benzoate (**182**) (4 g, 24.21 mmol) in dry THF (20 ml) at rt under Ar. The reaction mixture was heated to reflux for 24 h. The solution remaining was evaporated *in vacuo* to dryness. Citric acid (aq, sat. solution, 20 ml) was added and the mixture was extracted with ethyl acetate (2 x 60 ml). The organic extracts were collected, dried over Na_2SO_4, and the solvent removed under reduced pressure. The pure compound **183** was obtained after trituration with a solution (20 ml) of ethyl acetate:hexanes 1:9 as a white solid (4.2 g, 16 mmol, 67%), mp 141-143 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (3H, t, *J* 7.6 Hz, $CH_2C\underline{H}_3$), 1.54 (9H, s, $C(C\underline{H}_3)_3$), 4.35 (2H, q, *J* 7.2 Hz, $C\underline{H}_2CH_3$), 6.67 (1H, bs, NH), 7.42 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.97 (2H, d, *J* 8.8 Hz, 2 x CH, Ar).

***4-(Allyl-tert-butoxycarbonylamino)benzoic acid ethyl ester* (184)**

Sodium hydride (60% dispersion in mineral oil, 1.2 g, 29.2 mmol) was placed under Ar in a 100 ml two-necked flask, washed with dry THF (2 x 7 ml), and suspended in dry THF (20 ml). The mixture was cooled to 0 °C and **183** (2.4 g, 9.13 mmol) was added portionwise. After stirring for 15 min at room temperature, a solution of allyl bromide (2.64 g, 21.9 mmol, 2.4 eq) in dry THF (10 ml) was added drop wise. After stirring at room temperature for 48 h, the resulting mixture was quenched by slow dropwise addition of water (10 ml), and the resulting mixture was extracted with ethyl acetate (2 x 30 ml). The organic extracts were collected, dried over Na_2SO_4 and the solvent was evaporated to dryness. Hexane (50 ml) was added and the formed white solid was separated by filtration. The filtrate was evaporated *in vacuo* to give the pure product as a yellow oil (1.6 g, 5.28 mmol, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (3H, t, *J* 7.2 Hz, $CH_2C\underline{H}_3$), 1.46 (9H, s, $C(C\underline{H}_3)_3$), 4.26 (1H, t, *J* 1.6 Hz, CH, $NC\underline{H}_2CHCH_2$), 4.28 (1H, t, *J* 1.6 Hz, CH, $NHC\underline{H}_2CHCH_2$), 4.36 (2H, q, *J* 7.2 Hz, $C\underline{H}_2CH_3$), 5.13-5.14 (1H, m, CH, $NCH_2CHC\underline{H}_2$), 5.15-5.18 (1H, m, CH, $NCH_2CHC\underline{H}_2$), 5.91 (1H, qt, *J* 5.2, 9.0 Hz, CH, $NCH_2C\underline{H}CH_2$), 7.32 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.98 (2H, d, *J* 8.6 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.55 ($CH_2\underline{C}H_3$), 29.57 ($C(\underline{C}H_3)_3$), 52.73 ($N\underline{C}H_2CHCH_2$), 61.10 ($\underline{C}H_2CH_3$), 81.28 29.91 ($\underline{C}(CH_3)_3$), 116.76 ($NCH_2CH\underline{C}H_2$), 125.34 ($NCH_2\underline{C}HCH_2$), 127.33 (C, Ar), 130.21 (2 x CH, Ar), 134.16 (2 x CH, Ar), 147.23 (C), 154.09 (C=O), 166.36 (C=O). ν_{max} (oil)/(cm^{-1}) 2925 (md), 1703 (st), 1606 (st), 1366 (st), 1271 (st), 1169 (st), 1148 (st), 1103 (st). MS *m/z* (**API-ES**): found 306 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 306.1703 $(M+H)^+$, calculated for $C_{17}H_{24}NO_4$ 306.1705

Ethyl 4-(acryloylallylamino)benzoate **(185)**

To a solution of **184** (1.23 g, 4.07 mmol) in DCM (10 ml) TFA (10 ml) was added. After stirring for 2 h at room temperature, the solvent was distilled under reduced pressure to give a brown oil. The crude allylamine was dissolved as obtained in dry DCM (20 ml) under Ar. Triethylamine (1.650 g, 16.31mmol) was added followed by acryloyl chloride (0.405 g, 4.48 mmol) at room temperature. After stirring for 4 h at room temperature, the resulting mixture was quenched with water (10 ml) and extracted with DCM (2 x 20 ml). The organic extracts were collected, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Chromatography on silica gel (80:20 hexane:ethyl acetate, R_f 0.28) afforded **185** as a yellow oil (0.467 g, 1.8 mol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (3H, t, *J* 7.0 Hz, $CH_2C\underline{H}_3$), 4.39 (2H, d, *J* 7.0 Hz, $C\underline{H}_2CH_3$), 4.41-4.43 (2H, m, 2 x CH, $NC\underline{H}_2CHCH_2$), 5.08-5.16 (2H, m, 2 x CH, $NCH_2CHC\underline{H}_2$), 5.58 (1H, dd, *J* 1.8, 13.2 Hz, $COCHC\underline{H}_2$), 5.88 (1H, qt, *J* 6.2, 9.0 Hz, CH, $NCH_2C\underline{H}CH_2$), 6.04 (1H, dd, *J* 10.0, 16.8 Hz, $COC\underline{H}CH_2$), 6.41 (1H, dd, *J* 2.0, 16.8 Hz, CH, $COCHC\underline{H}_2$), 7.23 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 8.07 (2H, d, *J* 8.4 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.53 ($CH_2\underline{C}H_3$), 52.47 ($N\underline{C}H_2CHCH_2$), 61.47 ($\underline{C}H_2CH_3$), 118.36 ($NCH_2CH\underline{C}H_2$), 127.96 (2 x CH, Ar), 128.65 ($COCH\underline{C}H_2$), 128.72 ($NCH_2\underline{C}HCH_2$), 129.86 (C, Ar), 131.01 (2 x CH, Ar), 132.90 ($CO\underline{C}HCH_2$), 146.24 (C, Ar), 165.29 (C=O), 165.92 (C=O). ν_{max} (oil)/(cm^{-1}) 2981 (st), 1714 (st), 1660 (st), 1603 (st), 1403 (st), 1271 (st), 1101 (st). MS *m/z* (**API-ES**): found 260 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 260.1287 $(M+H)^+$, calculated for $C_{15}H_{18}NO_3$ 260.1287.

Ethyl 4-(2-oxo-2,5-dihydropyrrol-1-yl)benzoate **(186)**[386]

To a solution of **185** (0.057 g, 0.22 mmol) in dry toluene (11 ml) Grubbs catalyst (2nd generation, 0.0093 g, 0.0110 mmol, 5 mol %) was added at room temperature under Ar. After stirring for 1 h at 80 °C, the solvent was removed under reduced pressure. Chromatography on silica gel using the FlashMaster 3 purification station (60:40 hexane:ethyl acetate) afforded **186** as an off white solid (0.047 g, 0.20 mmol, 92%), mp 72-74 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (3H, t, *J* 6.8 Hz, $C\underline{H}_2CH_3$), 4.37 (2H, d, *J* 6.8 Hz, $C\underline{H}_2CH_3$), 4.49 (2H, t, *J* 1.8 Hz, $NC\underline{H}_2CHCHCO$), 6.30 (1H, dt, *J* 1.8, 6.0 Hz, $NCH_2CHC\underline{H}CO$), 7.23 (1H, dt, *J* 1.8, 6.0 Hz, $NCH_2CHC\underline{H}CO$), 7.32 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 8.06 (2H, d, *J* 8.8 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.58 ($CH_2\underline{C}H_3$), 53.13 ($N\underline{C}H_2CHCHCO$), 61.09 ($\underline{C}H_2CH_3$), 117.58 (2 x CH, Ar), 125.86 (C, Ar), 129.55 ($NCH_2CH\underline{C}HCO$), 131.04 (2 x CH, Ar), 142.87 ($NCH_2\underline{C}HCHCO$), 143.20 (C, Ar), 166.37 (C=O), 170.53 (C=O). ν_{max} (solid)/(cm^{-1}) 2990 (md), 1733 (st), 1652 (st), 1507 (st), 1365 (st), 1222 (st). MS *m/z* (**API-ES**): found 232 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 232.0971 $(M+H)^+$, calculated for $C_{13}H_{14}NO_3$ 232.0974.

Methyl 4-(4-nitromethyl-2-oxopyrrolidin-1-yl)benzoate **(187)**. This was prepared from corresponding amide **186** (0.039 g, 0.168 mmol) in a similar manner as described for

preparation of **144l**. Pure **187** was obtained as a yellow oil without purification (0.045 g, 0.154 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (1H, t, *J* 7.2 Hz, $CH_2C\underline{H_3}$), 2.47 (1H, dd, *J* 7.6, 17.6 Hz, CH, H-3'), 2.91 (1H, dd, *J* 8.8, 17.2 Hz, CH, H-3'), 3.31 (1H, septuplet, *J* 7.4 Hz, CH, H-4'), 3.75 (1H, dd, *J* 6.6, 9.8 Hz, CH, H-5'), 4.15 (1H, dd, *J* 7.8, 10.2 Hz, CH, H-5'), 4.38 (1H, q, *J* 7.2 Hz, $C\underline{H_2}CH_3$), 4.55 (1H, dd, *J* 7.8, 13.0 Hz, CH, CH_2NO_2), 4.60 (1H, dd, *J* 6.6, 13.4 Hz, CH_2NO_2), 7.67 (2H, d, *J* 9.2 Hz, 2 x CH, Ar), 8.04 (2H, d, *J* 9.2 Hz, 2 x CH, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.55 ($CH_2\underline{C}H_3$), 29.75 ($\underline{C}HCH_2NO_2$), 36.23 (CH_2), 51.53 (CH_2), 61.25 ($\underline{C}H_2CH_3$), 76.94 ($CH\underline{C}H_2NO_2$), 119.05 (2 x CH, Ar), 126.87 (C, Ar), 130.79 (2 x CH, Ar), 142.55 (C, Ar), 166.20 (C=O), 171.64 (C=O). ν_{max} (oil)/(cm^{-1}) 2981 (md), 1699 (st), 1605 (st), 1549 (st), 1270 (st). MS *m/z* (**API-ES**): found 293 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 293.1136 $(M+H)^+$, calculated for $C_{14}H_{17}N_2O_5$ 293.1137.

***2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonyl chloride* (189)**[392]

Phosphorus oxychloride (27.17 g, 177.2 mmol) was added to a mixture of 5-isatinsulfonic acid sodium salt dihydrate (**188**) (10.1 g, 35.5 mmol) in of tetramethylene sulfone (50 ml). The resulting mixture was stirred at 60 °C for 3 h. After cooling to 0 °C, water (120 ml) was added. The green precipitate was filtered, dissolved in ethyl acetate (200 ml) and washed with water (150 ml). The organic extracts were collected, dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to provide a green solid. The pure compound **179** was obtained after recrystallization from ethyl acetate/hexane 1:1 as yellow solid (5.9 g, 21.1 mmol, 68 %), mp 200-202 °C. ^{1}H NMR (400 MHz, $CDCl_3/CD_3CN$ 1:1) δ 7.22 (1H, d, *J* 8.4 Hz, H-7), 8.16 (1H, d, *J* 2.0 Hz, H-4), 8.23 (1H, dd, *J* 2.0 8.4Hz, H-6), 9.47 (1H, s, NH).

***2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide* (190r)**[392]

A mixture of dimethylamine (2M solution in THF) (0.334 ml, 0.668 mmol) and DIPEA (0.139 g, 1.02 mmol) was added to a solution of **189** (0.126 g, 0.514 mmol) in anhydrous THF (4 ml) at 0 °C under Ar. The reaction mixture was stirred overnight at room temperature, and the mixture was poured into water (5 ml). The product was extracted with ethyl acetate (3 x 10 ml). The organic extracts were collected, dried over Na_2SO_4 and the solvent removed under reduced pressure. The pure compound **190r** was obtained after trituration with ethyl acetate (5 ml) as a yellow solid (0.90 g, 0.354 mmol, 68%), mp 150-152 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60 (6H, s, $N(CH_3)_2$), 7.09 (1H, d, *J* 8.3 Hz, H-7), 7.68 (1H, d, *J* 2.0 Hz, H-4), 7.91 (1H, dd, *J* 8.3, 2.0 Hz, H-6), 11.44 (1H, s, NH). ν_{max} (solid)/(cm^{-1}) 3288 (md), 1783 (md), 1746 (st), 1705 (st) , 1623 (st), 1323 (st), 1135 (st), 1048 (st). MS *m/z* (**API-ES**): found 355 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 255.0443 $(M+H)^+$, calculated for $C_{10}H_{11}N_2O_4S$ 255.0440.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfoniamide **(190a)**. This was obtained from **189** (0.229 g, 0.946 mmol) and ammonia (2 M solution in ethanol) (0.603 ml, 1.21 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190a** was obtained after trituration with ethyl acetate as a yellow solid (0.099 g, 0.458 mmol, 47%), mp 200 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.02 (1H, d, *J* 8.2 Hz, H-7), 7.38 (2H, s, NH_2), 7.82 (1H, d, *J* 1.8 Hz, H-4), 7.95 (1H, dd, *J* 1.8, 8.2 Hz, H-6), 11.35 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 112.90 (CH, Ar), 118.50 (C, Ar), 122.44 (CH, Ar), 135.74 (CH, Ar), 139.13 (C, Ar), 153.34 (C, Ar), 160.26 (C=O), 184.00 (C=O). ν_{max} (solid)/(cm^{-1}) 3282 (md), 3120 (md), 1765 (md), 1747 (st), 1704 (st) (C=O), 1624 (st), 1343 (st), 1113 (st), 998 (st). MS *m/z* (**API-ES**): found 227 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 227.0125 $(M+H)^+$, calculated for $C_8H_7N_2O_4S$ 227.0127.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid (2-dimethylamino-ethyl)amide **(190b)** This was obtained from **189** (0.218 g, 0.889 mmol) and *N*,*N*-dimethylethylenediamine (0.101 g, 1.156 mmol) in a similar manner as described for preparation of **190r**. The crude product was used in the next step without further purification.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid propylamide **(190c)**. This was obtained as from **189** (0.256 g, 1.044 mmol) and propylamine (0.080 g, 1.358 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190c** was obtained after trituration with ethyl acetate as a yellow solid (0.125 g, 0.460 mmol, 45%), mp 242-244 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.77 (3H, t, *J* 7.4 Hz, $CH_2CH_2C\underline{H_3}$), 1.39 (2H, sex, *J* 7.2 Hz, $CH_2C\underline{H_2}CH_3$), 2.65 (2H, q, *J* 6.7 Hz, $C\underline{H_2}CH_2CH_3$), 7.04 (1H, d, *J* 8.4 Hz, H-7), 7.58 (1H, t, *J* 6.0 Hz, $HNSO_2$), 7.75 (1H, d, *J* 1.8 Hz, H-4), 7.92 (1H, dd, *J* 1.8, 8.4 Hz, H-6), 11.38 (1H, s, NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ 11.84 ($CH_2CH_2\underline{C}H_3$), 23.04 ($CH_2\underline{C}H_2CH_3$), 45.02 ($\underline{C}H_2CH_2CH_3$), 113.14 (CH, Ar), 118.78 (C, Ar), 123.09 (CH, Ar), 135.40 (C, Ar), 136.71 (CH, Ar), 153.73 (C, Ar), 160.21 (C=O), 183.86 (C=O). ν_{max} (solid)/(cm^{-1}) 3289 (md), 3119 (md), 1766 (md), 1747 (st), 1707 (st), 1620 (st), 1319 (st), 1150 (st), 1064 (st). MS *m/z* (**API-ES**): found 269 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 269.0591 $(M+H)^+$, calculated for $C_{11}H_{13}N_2O_4S$ 269.0596.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide **(190d)**. This was obtained from **189** (0.320 g, 1.306 mmol) and isopropylamine (0.100 g, 1.698 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190r** was obtained after trituration with ethyl acetate as a yellow solid (0.244 g, 0.910 mmol, 70%), mp 184-186 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (6H, d, *J* 6.8 Hz, $CH(\underline{CH_3})_2$), 3.11 (1H, sept, *J* 6.8 Hz, $C\underline{H}(CH_3)_2$), 7.04 (1H, d, *J* 8.2 Hz, H-7), 7.58 (1H, d, *J* 7.2 Hz, $HNSO_2$), 7.77 (1H, d, *J* 1.8 Hz, H-4), 7.94 (1H, dd, *J* 1.8, 8.2 Hz, H-6), 11.38 (1H, s, NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ 23.90 ($CH(\underline{CH_3})_2$), 45.99 ($\underline{C}H(CH_3)_2$), 113.15 (CH, Ar), 118.73 (C, Ar), 123.00 (CH,

Ar), 136.53 (CH, Ar), 136.55 (C, Ar), 153.57 (C, Ar), 160.20 (C=O), 183.90 (C=O). ν_{max} (solid)/(cm^{-1}) 3294 (md), 3115 (md), 1764 (md), 1748 (st), 1706 (st), 1619 (st), 1322 (st), 1114 (st), 1063 (st), 980 (st). MS *m/z* (**API-ES**): found 269 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 269.0594 $(M+H)^+$, calculated for $C_{11}H_{13}N_2O_4S$ 269.0596.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid (2-methoxy-ethyl)amide **(190e)**. This was obtained from **189** (0.308 g, 1.257 mmol) and 2-methoxyethylamine (0.103 g, 1.382 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190e** was obtained after trituration with ethyl acetate:DCM (1:3, v/v) as a yellow solid (0.221 g, 0.778 mmol, 62%), mp 120 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.87 (2H, q, *J* 5.6 Hz, $C\underline{H}_2CH_2OCH_3$), 3.14 (3H, S, OCH_3), 3.28 (2H, t, *J* 5.8 Hz, $CH_2C\underline{H}_2OCH_3$), 7.04 (1H, d, *J* 8.3 Hz, H-7), 7.75 (1H, t, *J* 6.0 Hz, $HNSO_2$), 7.78 (1H, d, *J* 1.7 Hz, H-4), 7.93 (1H, dd, *J* 1.7, 8.3 Hz, H-6), 11.38 (1H, s, NH).^{13}C NMR (400 MHz, DMSO-d_6) δ 42.86 ($\underline{C}H_2CH_2OCH$), 58.56 (OCH_3), 71.15 ($CH_2\underline{C}H_2OCH_3$), 113.08 (CH, Ar), 118.69 (C, Ar), 123.22 (CH, Ar), 135.49 (C, Ar), 136.73 (CH, Ar), 153.75 (C, Ar), 160.21 (C=O), 183.88 (C=O). ν_{max} (solid)/(cm^{-1}) 3275 (md), 3119 (md), 1765 (md), 1746 (st), 1706 (st), 1619 (st), 1316 (st), 1147 (st), 1122 (st), 1062 (st). MS *m/z* (**API-ES**): found 285 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 285.0544 $(M+H)^+$, calculated for $C_{11}H_{13}N_2O_5S$ 285.0545

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid sec-butylamide **(190f)**. This was obtained from **189** (0.23 g, 0.951 mmol) and sec-butylamine (0.076 g, 1.046 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190f** was obtained after trituration with ethyl acetate as a yellow solid (0.080 g, 0.283 mmol, 30%), mp 208-210 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.71 (3H, t, *J* 7.6 Hz, $NCHCH_2C\underline{H}_3$), 0.87 (3H, d, *J* 6.8 Hz, $NCHC\underline{H}_3$), 1.29 (2H, quint, t, *J* 7.2 Hz, $NCHC\underline{H}_2CH_3$), 3.01 (1H, quint, *J* 6.7 Hz, $NC\underline{H}CH_2CH_3$), 7.03 (1H, d, *J* 8.2 Hz, H-7), 7.53 (1H, d, *J* 7.2 Hz, $HNSO_2$), 7.75 (1H, s, H-4), 7.93 (1H, d, *J* 8.2 Hz, H-6), 11.36 (1H, s, NH). MS *m/z* (**API-ES**): found 283 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 283.0749 $(M+H)^+$, calculated for $C_{12}H_{15}N_2O_4S$ 283.0753.

5-(Morpholine-4-sulfonyl)-1H-indole-2,3-dione **(190g)**[395]

A solution of **189** (0.211 g, 0.861 mmol) and morpholine (0.187 g, 2.139 mmol, 2.5 eq), in anhydrous DCM (7 ml) and anhydrous chloroform (1 ml) was stirred for 3h at room temperature under Ar. The yellow precipitate was collected by filtration and dried under vacuum. The crude product was used in the next step without further purification.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid (tetrahydrofuran-2-ylmethyl)amide **(190h)**. This was obtained from **189** (0.236 g, 0.960 mmol) and tetrahydrofurfurylamine (0.107 g, 1.05 mmol) in a similar manner as described for preparation of **190r**. The pure

compound **190h** was obtained after trituration with ethyl acetate ethyl acetate/hexane (3:2 v/v) as a yellow solid (0.199 g, 0.633 mmol, 66%), mp 180 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46-1.53 (1H, m), 1.69-1.85 (3H, m), 2.69-2.78 (2H, m), 3.51-3.67 (2H, m), 3.74-3.80 (1H, m), 7.04 (1H, d, *J* 8.0 Hz, H-7), 7.74 (1H, t, *J* 6.2 Hz, $NHSO_2$), 7.79 (1H, d, *J* 1.8 Hz, H-4), 7.93 (1H, dd, *J* 1.8, 8.2 Hz, H-6), 11.38 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.76 ($OCH_2\underline{CH_2}$), 29.0 ($NCH_2CH(\underline{CH_2})O$), 47.21 ($NC\underline{H_2}CHO$), 67.97 ($OC\underline{H_2}CH_2$), 77.60 ($NCH_2C\underline{H}O$) 113.09 (CH, Ar), 118.70 (C, Ar), 123.25 (CH, Ar), 135.49 (C, Ar), 136.75 (CH, Ar), 153.75 (C, Ar), 160.21 (C=O), 183.89 (C=O). ν_{max} (solid)/(cm^{-1}) 3161 (md), 1766 (md), 1747 (st), 1707 (st), 1619 (st), 1316 (st), 1062 (st). MS *m/z* (**API-ES**): found 311 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 311.0700 $(M+H)^+$, calculated for $C_{13}H_{15}N_2O_5S$ 311.0702

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid (furan-2-ylmethyl)amide **(190i)**. This was obtained from **189** (0.292 g, 1.191 mmol) and furfurylamine (0.127 g, 1.311 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190i** was obtained after trituration with ethyl acetate ethyl acetate/hexane 3:2 as a yellow solid (0.349 g, 1.140 mmol, 95%), mp 120 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.98 (2H, d, *J* 6.0 Hz, CH_2), 6.17 (1H, d, *J* 2.6 Hz, CH, Ar), 6.27 (1H, dd, *J* 1.2, 2.6 Hz, CH, Ar), 6.99 (1H, d, *J* 8.5 Hz, H-7), 7.45 (1H, dd, *J* 1.2, 2.6 Hz, CH, Ar), 7.71 (1H, d, *J* 1.9 Hz, H-4), 7.88 (1H, dd, *J* 1.9, 8.5 Hz, H-6), 8.18 (1H, t, *J* 6.0 Hz, $HNSO_2$), 11.40 (1H, s, NH). ^{13}C NMR (400 MHz, DMSO-d_6) δ 39.96 (CH_2), 108.84 (CH, Ar), 111.06 (CH, Ar), 113.06 (CH, Ar), 118.51 (C, Ar), 123.30 (CH, Ar), 135.54 (C, Ar), 136.79 (CH, Ar), 143.28 (CH, Ar), 150.93 (C, Ar), 153.74 (C, Ar), 160.17 (C=O), 183.86 (C=O). ν_{max} (solid)/(cm^{-1}) 3270 (md), 3114 (md), 1766 (md), 1746 (st), 1706 (st), 1619 (st), 1321 (st), 1148 (st), 1064 (md), 726 (st). MS *m/z* (**API-ES**): found 307 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 307.0386 $(M+H)^+$, calculated for $C_{13}H_{11}N_2O_5S$ 307.0389.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid (thiophen-2-ylmethyl)amide **(190j)**. This was obtained from **189** (0.202 g, 0.824 mmol) and 2-thiophenemethylamine (0.121 g, 1.071 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190j** was obtained after trituration with ethyl acetate as a yellow solid (0.129 g, 0.400 mmol, 49%), mp 180 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.16 (2H, d, *J* 6.1 Hz, CH_2), 6.86-6.88 (2H, m, H-3' & H-5') 7.02 (1H, d, *J* 8.2 Hz, H-7), 7.37 (1H, dd, *J* 2.0, 4.4 Hz, H-4'), 7.74 (1H, d, *J* 2.0 Hz, H-4), 7.92 (1H, dd, *J* 2.0, 8.2, 2.0 Hz, H-6), 8.27 (1H, t, *J* 6.1 Hz, $HNSO_2$), 11.40 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.80 (CH_2), 113.10 (CH, Ar), 118.61 (C, Ar), 123.30 (CH, Ar), 126.46 (CH, Ar), 126.86 (CH, Ar), 127.37 (CH, Ar), 135.41 (C, Ar), 136.82 (C, Ar), 140.99 (CH, Ar), 153.78 (C, Ar), 160.18 (C=O), 183.77 (C=O). ν_{max} (solid)/(cm^{-1}) 3267 (md), 3120 (md), 1771 (md), 1745 (st), 1705 (st), 1617 (st),

1319 (st), 1145 (st), 1053 (md), 750 (st). MS *m/z* (**API-ES**): found 323 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 323.0157 $(M+H)^+$, calculated for $C_{13}H_{11}N_2O4S_2$ 323.0160

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid 3-methoxybenzylamide **(190k)**. This was obtained from **189** (0.206 g, 1.093 mmol) and 3-methoxybenzylamine (0.149 g, 1.093 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190k** was obtained after trituration with ethyl acetate as a yellow (0.157 g, 0.453 mmol, 54%), mp 215-217 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.65 (3H, s, OCH_3), 3.95 (2H, d, *J* 6.6 Hz, CH_2), 6.72- 6.77 (3H, m, ArH), 6.98 (1H, d, *J* 8.2 Hz, H-7), 7.11-715 (1H, m, ArH), 7.68 (1H, d, *J* 1.6 Hz, H-4), 7.89 (1H, dd, *J* 1.6, 8.2, Hz, H-6), 8.15 (1H, t, *J* 6.6 Hz, $HNSO_2$), 11.39 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.80 (CH_2), 55.60 (OCH_3), 113.05 (CH, Ar), 113.26 (CH, Ar), 113.79 (CH, Ar), 118.48 (C, Ar), 120.52 (CH, Ar), 123.27 (CH, Ar), 129.99 (CH, Ar), 135.64 (C, Ar), 136.78 (C, Ar), 139.42 (CH, Ar), 153.65 (C, Ar), 159.83 (C, Ar), 160.15 (C=O), 183.73 (C=O). ν_{max} (solid)/(cm^{-1}) 3274 (md), 3116 (md), 1761 (md), 1744(st), 1705 (st), 1619 (st), 1489 (st), 1319 (st), 1147 (st), 852 (st). MS *m/z* (**API-ES**): found 374 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 374.0700 $(M+H)^+$, calculated for $C_{16}H_{15}N_2O_5S$ 347.0702.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-methoxybenzylamide **(190l)**. This was obtained from **189** (0.203 g, 0.828 mmol) and 4-methoxybenzylamine (0.147 g, 1.077 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190l** was obtained after trituration ethyl acetate as a yellow solid (0.072 g, 0.120 mmol, 25%), mp 230 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.66 (3H, s, OCH_3), 3.89 (2H, d, *J* 6.1 Hz, CH_2), 6.77 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 6.98 (1H, d, *J* 8.0 Hz, H-7), 7.08 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.65 (1H, d, *J* 2.0 Hz, H-4), 7.88 (1H, dd, *J* 8.0, 2.0 Hz, H-6), 8.06 (1H, t, *J* 6.1 Hz, $HNSO_2$), 11.39 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.43 (CH_2), 55.69 (OCH_3), 113.05 (CH, Ar), 114.29 (2 x CH, Ar), 118.52 (C, Ar), 123.30 (CH, Ar), 129.72 (2 x CH, Ar), 129.57 (C, Ar), 135.73 (CH, Ar), 136.79 (C, Ar), 153.63 (C, Ar), 159.12 (C, Ar), 160.17 (C=O), 183.78 (C=O). ν_{max} (solid)/(cm^{-1}) 3270 (md), 3114 (md), 1763 (md), 1745(st), 1707 (st), 1618 (st), 1493 (st), 1319 (st), 1148 (st), 850 (st). MS *m/z* (**API-ES**): found 374 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 374.0699 $(M+H)^+$, calculated for $C_{16}H_{15}N_2O_5S$ 347.0702.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid benzylmethylamide **(190m)**. This was obtained from **189** (0.227 g, 0.926 mmol) and *N*-methylbenzylamine (0.145 g, 1.203 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190m** was obtained after trituration with ethyl acetate as a yellow solid (0.155 g, 0.469 mmol, 47%), mp 170-172 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (3H, s, NCH_3), 4.13 (2H, s, CH_2), 7.10 (1H, d, *J* 8.4 Hz, H-7), 7.25-7.37 (5H, m, ArH), 7.79 (1H, d, *J* 1,9 Hz, H-4), 8.01 (1H, dd, *J*

8.4, 1.9 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ 35.17 (CH_3), 54.01 (CH_2), 113.52 (CH, Ar), 118.90 (C, Ar), 123.87 (CH, Ar), 128.42 (CH, Ar), 128.84 (2 x CH, Ar), 129.28 (2 x CH, Ar), 131.71 (C, Ar), 136.62 (C, Ar), 137.53 (CH, Ar), 154.35 (C, Ar), 160.14 (C=O), 183.65 (C=O). ν_{max} (solid)/(cm^{-1}) 3124 (md), 1763 (md), 1746 (st), 1707 (st), 1629 (st), 1312 (st), 1154 (st), 780 (st). MS *m/z* (**API-ES**): found 331 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 331.0751 $(M+H)^+$, calculated for $C_{16}H_{15}N_2O_4S$ 331.0753.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid (pyridin-4-ylmethyl)amide **(190n)**. This was obtained from **189** (0.225 g, 0.918 mmol) and 4-(aminomethyl)pyridine (0.129 g, 1.193 mmol) in a similar manner as described for preparation of **190n**. The crude was used in the next step without further purification.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid (pyridin-2-ylmethyl)amide **(198o)**. This was obtained from **189** (0.205 g, 0.836 mmol) and 2-(aminomethyl)pyridine (0.200 g, 1.877 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190o** was obtained after trituration with ethyl acetate as a yellow solid (0.145 g, 0.457 mmol, 58%), mp 140 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.06 (2H, d, *J* 6.2 Hz, CH_2), 6.99 (1H, d, *J* 8.4 Hz, H-7), 7.19-7.22 (1H, m, ArH), 7.32 (1H, d, *J* 7.7 Hz, ArH), 7.70 (1H, td, *J* 1.7, 7.7 Hz, ArH), 7.74 (1H, d, *J* 2.0 Hz, H-4), 7.91 (1H, dd, *J* 2.0, 8.4 Hz, H-6), 8.28 (1H, t, *J* 6.2 Hz, $HNSO_2$), 8.39-8.40 (1H, m, ArH), 11.39 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 48.64 (CH_2), 113.05 (CH, Ar), 118.62 (C, Ar), 122.48 (CH, Ar), 123.09 (CH, Ar), 123.34 (CH, Ar), 135.39 (C, Ar), 136.84 (CH, Ar), 137.44 (CH, Ar), 148.48 (CH, Ar), 153.75 (C, Ar), 157.51 (C, Ar), 160.19 (C=O), 183.78 (C=O). ν_{max} (solid)/(cm^{-1}) 3132 (md), 1755 (md), 1731 (st), 1699 (st), 1621 (st), 1322 (st), 1201 (st), 743 (st). MS *m/z* (**API-ES**): found 318 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 318.0547 $(M+H)^+$, calculated for $C_{14}H_{12}N_3O_4S$ 318.0549

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide **(190p)**. This was obtained from **189** (0.121 g, 0.869 mmol) and 4-chlorobenzylamine (0.159 g, 1.130 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190p** was obtained after trituration with ethyl acetate as a yellow solid (0.175 g, 0.502 mmol, 57%), mp 250 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.97 (2H, d, *J* 6.2 Hz, CH_2), 7.00 (1H, d, *J* 8.1 Hz, H-7), 7.20 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.29 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.68 (1H, d, *J* 1.9 Hz, H-4), 7.89 (1H, dd, *J* 8.1, 1.9 Hz, H-6), 8.22 (1H, t, *J* 6.2 Hz, $HNSO_2$), 11.41 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.08 (CH_2), 113.10 (CH, Ar), 118.57 (C, Ar), 123.24 (CH, Ar), 128.85 (2 x CH, Ar), 130.20 (2 x CH, Ar), 132.40 (C, Ar), 135.52 (C, Ar), 136.76 (C, Ar), 137.12 (CH, Ar), 153.75 (C, Ar), 160.15 (C=O), 183.72 (C=O). ν_{max} (solid)/(cm^{-1}) 3283 (md), 3124 (md), 1767 (md), 1739 (st), 1705 (st), 1623 (st), 1330 (st), 1113 (st), 904 (st). MS *m/z* (**API-ES**): found 351 $(M^{35}C+H)^+$ (100%),

353 ($M^{37}C+H)^+$ (100%). HRMS *m/z* (**API-ES**): 351.0204 $(M+H)^+$, calculated for $C_{15}H_{12}ClN_2O_4S$ 351.0206.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid benzylamide **(190q)**. This was obtained from **189** (0.553 g, 2.350 mmol) and benzylamine (0.326 g, 3.051 mmol) in a similar manner as described for preparation of **190r**. The pure compound **190q** was obtained after trituration with ethyl acetate as an orange solid (0.545 g, 1.735 mmol, 74%), mp 225-227 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.96 (2H, d, *J* 5.6 Hz, CH_2), 7.01 (1H, d, *J* 8.0 Hz, H-7), 7.20-7.25 (5H, m, ArH), 7.73 (1H, s, H-4), 7.93 (1H, d, *J* 8.0 Hz, H-6), 8.17 (1H, bt, *J* 5.6 Hz, $NHSO_2$), 11.41 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.83 (CH_2), 113.1 (CH, Ar), 118.61 (C, Ar), 123.26 (CH, Ar), 127.81 (CH, Ar), 128.33 (2 x CH, Ar), 128.94 (2 x CH, Ar), 135.52 (C, Ar), 136.79 (CH, Ar), 138.00 (C, Ar), 153.71 (C, Ar), 160.17 (C=O), 183.77 (C=O). ν_{max} (solid)/(cm^{-1}) 3265 (st), 3122 (md), 1764 (md), 1739 (st), 1705 (st), 1628 (st), 1323 (st), 1152 (st), 785 (st). MS *m/z* (**API-ES**): found 317 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): 317.0593 $(M+H)^+$, calculated for $C_{15}H_{13}N_2O_4S$ 317.0596.

1-Ethyl-2,3-dioxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide **(190t)**

Ethyl bromide (0.173 g, 1.592 mmol) was added to a solution of **190r** (0.043 g, 0.169 mmol 0.160 g) and NaH (60% suspension in mineral oil, 0.0101 g, 0.253 mmol) in anhydrous DMF (1 ml) at room temperature under Ar. After stirring overnight at room temperature under Ar, the reaction mixture was poured into water (4 ml). The mixture was extracted with ethyl acetate (3 x 10 ml), dried over Na_2SO_4 and the solvent was distilled under reduced pressure. Chromatography on silica gel (60:40 hexanes/ethyl acetate, R_f 0.40) afforded **190t** as a red solid (0039 g, 0.138 mmol, 82%), mp 165-167 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71 (3H, t, *J* 7.2 Hz, $CH_2\underline{CH_3}$), 2.74 (6H, s, $N(CH_3)_2$), 3.84 (2H, q, *J* 7.2 Hz, $\underline{CH_2}CH_3$), 7.06 (1H, d, *J* 8.0 Hz, H-7), 7.97 (1H, d, *J* 1.8 Hz, H-4), 8.02 (1H, dd, *J* 1.8, 8.0 Hz, H-6). ^{13}C NMR (400 MHz, $CDCl_3$) δ 12.72 ($CH_2\underline{C}H_3$), 35.69 (2 x CH_3), 38.07 ($\underline{C}H_2CH_3$), 110.50 (CH, Ar), 117.66 (C, Ar), 124.99 (CH, Ar), 131.96 (C, Ar), 137.91 (CH, Ar), 153.69 (C, Ar), 157.63 (C=O), 182.37 (C=O). ν_{max} (solid)/(cm^{-1}) 1771 (md), 1732 (st), 1707(st), 1619 (st), 1317 (st), 1138 (st), 1023 (st). MS *m/z* (**API-ES**): found 283 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): 283.0748 $(M+H)^+$, calculated for $C_{12}H_{15}N_2O_4S$ 283.0753.

1-Methyl-2,3-dioxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide **(190s)**. This was obtained from **190r** (0.092 g, 0.362 mmol) and iodomethane (0.102 g, 0.742 mmol) in a similar manner as described for preparation of **190t**. The compound was purified via chromatography on silica gel (hexanes/ethyl acetate 7:3) to give **190s** as a red solid (0.065 g, 0.242 g, 67%), mp 190-192 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.74 (6H, s, $N(CH_3)_2$), 3.33 (3H, s, NCH_3), 7.08 (1H, d, *J* 8.2 Hz, H-7), 7.98 (1H, d, *J* 2.0 Hz, H-4), 8.05 (1H, dd, *J* 2.0,

8.2 Hz. H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.62 (CH_3), 36.83 (2 x CH_3), 109.30 (CH, Ar), 116.26 (C, Ar), 123.51 (CH, Ar), 130.86 (C, Ar), 136.72 (CH, Ar), 153.15 (C, Ar), 156.76 (C=O), 180.80 (C=O). ν_{max} (solid)/(cm^{-1}) 1774 (md), 1738 (st), 1706 (st), 1619 (st), 1321 (st), 1138 (st), 1020 (st). MS *m/z* (**API-ES**): found 269 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): 269.0593 $(M+H)^+$, calculated for $C_{11}H_{13}N_2O_4S$ 269.0596.

1-Benzyl-2,3-dioxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide **(190u)**. This was obtained from **190r** (0.093 g, 0.366 mmol) and benzylbromide (0.187 g, 1.098 mmol) in a similar manner as described for preparation of **190t**. The compound was purified via chromatography on silica gel (hexanes/ethyl acetate 6:4) to give **190u** as a red solid (0.036 g, 0.104 mmol, 29%), mp 152-154 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71 (6H, s, N($\underline{CH_3}$)$_2$), 4.98 (2H, s, CH_2), 6.94 (1H, d, *J* 8.0 Hz, H-7), 7.33-7.40 (5H, m, 5 x CH, Ar), 7.91 (1H, dd, *J* 2.0, 8.0 Hz, H-6), 7.98 (1H, d, *J* 2.0 Hz, H-4). ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.04 (2 x CH_3), 44.70 (CH_2), 111.55 (CH, Ar), 117.73 (C, Ar), 124.86 (CH, Ar), 127.77 (2 x CH, Ar), 128.79 (2 x CH, Ar), 129.50 (CH, Ar), 132.27 (C, Ar), 133.96 (C, Ar), 137.78 (CH, Ar), 153.65 (C, Ar), 158.03 (C=O), 182.01 (C=O). ν_{max} (solid)/(cm^{-1}) 1773 (md), 1732 (st), 1706 (st), 1624 (st), 1319 (st), 1138 (st), 1032 (st). MS *m/z* (**API-ES**): found 345 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): 345.0905 $(M+H)^+$, calculated for $C_{17}H_{17}N_2O_4S$ 345.0909.

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide **($191a_2$)**

A solution of **190r** (0.09 g, 0.354 mmol) and 2-nitrophenylhydrazine (0.059 g, 0.389 mmol, 1.1 eq) in ethanol (8 ml) was stirred for 4 h at 80 °C in presence of HCl (aq 4M, 4 drops). Pure compound was obtained by filtration and dried *in vacuo* (0.083 g, 0.213 mmol, 60%), mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.62 (6H, s, $N(CH_3)_2$), 7.16 (1H, d, *J* 8.2 Hz, H-7), 7.22-7.24 (1H, m, ArH), 7.76 (1H, dd, *J* 2.0, 8.2 Hz, H-6), 7.81 (1H, t, *J* 7.9 Hz, ArH), 7.96 (1H, d, *J* 2.0 Hz, H-4), 8.23 (1H, dd, *J* 1.4, 7.9 Hz, ArH), 8.32 (1H, d, *J* 7.9 Hz, ArH), 11.62 (1H, s, HNCO), 14.25 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d_6) δ 38.36 (2 x CH_3), 111.85 (CH, Ar), 116.92 (CH, Ar), 119.65 (CH, Ar), 121.63 (C, Ar), 122.93 (CH, Ar), 126.44 (CH, Ar), 128.96 (C, Ar), 130.60 (CH, Ar), 132.39 (C, Ar), 133.90 (C, Ar), 137.23 (CH, Ar), 139.56 (C, Ar), 145.28 (C=N), 163.15 (C=O). ν_{max} (solid)/(cm^{-1}) 3182 (md), 1702 (md), 1613 (st) (C=O), 1567 (st), 1496 (st), 1332 (st), 1149 (st), 1120 (md). MS *m/z* (**API-ES**): found 390 $(M+H)^+$(100%). HRMS *m/z* (**API-ES**): found 390.0871 $(M+H)^+$, calculated for $C_{16}H_{16}N_5O_5S$ 390.0872; found 407.1139, $(M+NH_4)^+$, calculated for $C_{16}H_{19}N_6O_5S$ 407.1138.

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonicamide **($191a_1$)**. This was obtained as a yellow solid (0.090 g, 0.250 mmol, 58%) from **190a** (0.097 g, 0.429 mmol) and 2-nitrophenylhydrazine (0.072 g, 0.472 mmol) in a similar manner as described for

preparation of **191a$_2$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.03 (1H, d, *J* 8.2 Hz, H-7), 7.19-7.24 (1H, m, ArH), 7.30 (2H, s, NH$_2$), 7.77 (1H, dd, *J* 1.8, 8.2 Hz, H-6), 7.81-785 (1H, m, ArH), 8.07 (1H, d, *J* 1.8 Hz, H-4), 8.21-8.24 (2H, m, ArH), 11.53 (1H, s, HNCO), 14.24 (1H, s, HNN). ^{13}C NMR (400 MHz, DMSO-d$_6$) δ 111.44 (CH, Ar), 116.54 (CH, Ar), 118.15 (CH, Ar), 121.23 (C, Ar), 122.84 (C, Ar), 126.61 (CH, Ar), 128.64 (C, Ar), 132.87 (C, Ar), 133.94 (CH, Ar), 137.31 (CH, Ar), 138.83 (CH, Ar), 139.69 (C, Ar), 144.32 (C, Ar), 163.35 (C=O). ν_{max} (solid)/(cm^{-1}) 3431 (md), 3313 (md), 3182 (md), 1693 (st), 1615 (md), 1573 (md), 1494 (md), 1339 (st), 1156 (st). MS *m/z* (**API-ES**): found 379 (M+NH$_4$)$^+$ (100%). HRMS *m/z* (**API-ES**): found 379.0818 (M+NH$_4$)$^+$, calculated for $C_{14}H_{15}N_6O_5S$ 379.0825.

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid (pyridin-2-ylmethyl)amide **(191a$_{19}$)**. This was obtained as a yellow solid (0.095 g, 0.210 mmol, 58%) from **190o** (0.115 g, 0.361 mmol) and 2-nitrophenylhydrazine (0.061 g, 0.389 mmol) in a similar manner as described for preparation of **191a$_2$**, mp 275 °C (dec). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 4.08 (2H, d, *J* 6.2 Hz, CH$_2$), 7.07 (1H, d, *J* 8.1 Hz, H-7), 7.22 (2H, m, ArH) 7.35 (1H, d, *J* 7.6 Hz, ArH), 7.70 (1H, dt, *J* 2.0, 7.6 Hz, ArH), 7.73 (1H, dd, *J* 1.9, 8.1 Hz, H-6), 7.83 (1H, m, ArH), 7.99 (1H, d, *J* 1.9 Hz, H-4), 8.20 (1H, t, *J* 6.2 Hz, HNSO$_2$), 8.22-8.26 (2H, m, ArH), 8.39-8.40 (1H, m, ArH), 11.56 (1H, s, HNCO), 14.23 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 46.68 (CH$_2$), 111.58 (CH, Ar), 116.67 (CH, Ar), 118.97 (CH, Ar), 121.41 (C, Ar), 122.44 (CH, Ar), 122.87 (CH, Ar), 123.06 (CH, Ar), 126.57 (CH, Ar), 129.68 (CH, Ar), 132.63 (C, Ar), 133.94 (C, Ar), 134.93 (C, Ar), 137.29 (CH, Ar), 137.39 (CH, Ar), 137.67 (C, Ar), 144.76 (C, Ar), 149.38 (CH, Ar), 157.73 (C=N), 163.27 (C=O). ν_{max} (solid)/(cm^{-1}) 3274 (md), 1697 (md), 1609 (md), 1559 (md), 1492 (st), 1334 (st), 1148 (st), 733 (st). MS *m/z* (**API-ES**): found 453 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 453.0980 (M+H)$^+$, calculated for $C_{20}H_{17}N_6O_5S$ 453.0981.

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid benzyl-methylamide **(191a$_{17}$)**. This was obtained as a yellow solid (0.105 g, 0.225 mmol, 54%) from **190m** (0.140 g, 0.424 mmol) and 2-nitrophenylhydrazine (0.071 g, 0.466 mmol) in a similar manner as described for preparation of **191a$_2$**, mp 296-296 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 2.55 (3H, s, CH$_3$), 4.15 (2H, s, CH$_2$), 7.17 (1H, d, *J* 8.5 Hz, H-7), 7.21 (1H, m, ArH), 7.27-7.32 (5H, m, ArH), 7.78-7.82 (2H, m, ArH), 8.01 (1H, d, *J* 1.5 Hz, H-4), 8.23 (1H, dd, *J* 1.5, 8.5 Hz, H-6), 8.39 (1H, m, ArH), 11.63 (1H, s, HNCO), 14.26 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 35.24 (CH$_3$), 54.07 (CH$_2$), 111.98 (CH, Ar), 117.07 (CH, Ar), 119.51 (CH, Ar), 121.82 (C, Ar), 123.01 (CH, Ar), 126.52 (CH, Ar), 128.39 (2 x CH, Ar), 128.92 (2 x CH, Ar), 129.26 (2 x CH, Ar), 130.36 (CH, Ar), 131.21 (C, Ar), 132.43 (C, Ar), 134.02 (C, Ar), 136.79 (2 x CH, Ar), 137.27 (C, Ar), 139.65 (C, Ar),

145.28 (C=N), 163.24 (C=O). MS *m/z* (**API-ES**): found 466 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 466.1180 $(M+H)^+$, calculated for $C_{22}H_{20}N_5O_5S$ 466.1185.

***3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid 3-methoxybenzylamide* (191a$_{15}$)**. This was obtained as a yellow solid (0.092 g, 0.179 mmol, 45%) from **190k** (0.141 g, 0.407 mmol) and 2-nitrophenylhydrazine (0.068 g, 0.448 mmol) in a similar manner as described for preparation of **191a$_2$**, mp 265-267 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.62 (3H, s, OCH_3), 3.94 (2H, d, *J* 6.2 Hz, CH_2), 6.70 (1H, dd, *J* 8.0, 2 Hz, ArH), 6.75-6.78 (2H, m, ArH), 7.05 (1H, d, *J* 8.1 Hz, H-7), 7.12 (1H, t, *J* 8.0 Hz, ArH), 7.19-7.23 (1H, m, ArH), 7.71 (1H, dd, *J* 1.7, 8.1 Hz, H-6), 7.81 (1H, td, *J* 8.0 Hz, ArH), 7.94 (1H, d, *J* 1.7 Hz, H-4), 8.07 (1H, t, *J* 6.2 Hz, $HNSO_2$), 8.22 (2H, m, 2 x CH, Ar), 11.55 (1H, s, HNCO), 14.23 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.83 (CH_2), 55.58 (OCH_3), 111.59 (CH, Ar), 113.32 (CH, Ar), 113.69 (CH, Ar), 116.67 (CH, Ar), 118.97 (CH, Ar), 120.47 (CH, Ar), 121.37 (C, Ar), 122.88 (C, Ar), 126.57 (C, Ar), 129.62 (C, Ar), 129.91 (CH, Ar), 132.61 (CH, Ar), 133.97 (C, Ar), 135.20 (C, Ar), 137.27 (CH, Ar), 139.67 (CH, Ar), 139.71 (C, Ar), 144.68 (CH, Ar), 159.84 (C=N), 163.28 (C=O). ν_{max} (solid)/(cm^{-1}) 3150 (md), 1692 (md), 1613 (md), 1565 (md), 1490 (st), 1419 (md), 1323 (md), 1308 (md), 1262 (md), 1140 (st), 854 (md), 781 (st), 724 (st), 692 (st). MS *m/z* (**API-ES**): found 499 $(M+NH_4)^+$ (100%). HRMS *m/z* (**API-ES**): found 499.1392 $(M+NH_4)^+$, calculated for $C_{22}H_{29}N_6O_6S$ 499.1400.

***3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-methoxybenzylamide* (191a$_{16}$)**. This was obtained as a yellow solid (0.051 g, 0.103 mmol, 57%) from **190l** (0.063 g, 0182 mmol) and 2-nitrophenylhydrazine (0.030 g, 0.200 mmol) in a similar manner as described for preparation of **191a$_2$**, mp 261-263 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.62 (3H, s, OCH_3), 3.89 (2H, d, *J* 6.4 Hz, CH_2), 6.75 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.04 (1H, d, *J* 8.2 Hz, H-7), 7.06 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.19 (1H, t, *J* 7.8 Hz, ArH), 7.70 (1H, dd, *J* 1.6, 8.2 Hz, H-6), 7.80 (1H, t, *J* 7.8 Hz, Ar), 7.91 (1H, s, H-4), 7.96 (1H, t, *J* 6.4 Hz, $HNSO_2$), 8.20-8.23 (2H, m, ArH), 11.53 (1H, s, HNCO), 14.22 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.44 (CH_2), 55.67 (OCH_3), 111.59 (CH, Ar), 114.25 (2 x CH, Ar), 116.68 (CH, Ar), 118.98 (CH, Ar), 121.38 (C, Ar), 122.88 (CH, Ar), 126.58 (CH, Ar), 129.62 (CH, Ar), 129.69 (2 x CH, Ar), 129.99 (C, Ar), 132.66 (C, Ar), 133.96 (C, Ar), 135.25 (C, Ar), 137.31 (CH, Ar), 139.69 (C, Ar), 144.65 (C, Ar), 159.10 (C=N), 163.29 (C=O). ν_{max} (solid)/(cm^{-1}) 3285 (st), 3154 (md), 1689 (md), 1610 (st), 1491 (st), 1342 (md), 1307 (st), 1189 (st). MS *m/z* (**API-ES**): found 499 $(M+NH_4)^+$ (100%). HRMS *m/z* (**API-ES**): found 499.1387 $(M+NH4)^+$, calculated for $C_{22}H_{29}N_6O_6S$ 499.1400.

***3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide* (191a$_{25}$)**. This was obtained as a yellow solid (0.082 g, 0.169 mmol, 43%)

from **190p** (0.137 g, 0.393 mmol) and 2-nitrophenylhydrazine (0.066 g, 0.433 mmol) in a similar manner as described for preparation of **191a$_2$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 3.98 (2H, d, *J* 6.4 Hz, CH_2), 7.06 (1H, d, *J* 8.1 Hz, H-7), 7.23-7.27 (5H, m, ArH), 7.70 (1H, dd, *J* 1.7, 8.1 Hz, H-6), 7.82 (1H, t, *J* 8.4 Hz, ArH), 7.90 (1H, d, *J* 1.7 Hz, H-4), 8.13 (1H, t, *J* 6.4 Hz, $HNSO_2$), 8.23 (2H, m, ArH), 11.56 (1H, s, HNCO), 14.23 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 46.14 (CH_2), 111.62 (CH, Ar), 116.69 (CH, Ar), 118.94 (CH, Ar), 121.39 (C, Ar), 122.89 (CH, Ar), 126.58 (CH, Ar), 128.78 (2 x CH, Ar), 129.59 (CH, Ar), 130.18 (2 x CH, Ar), 132.38 (C, Ar), 132.58 (C, Ar), 133.95 (C, Ar), 135.11 (C, Ar), 137.32 (C, Ar), 137.34 (CH, Ar), 139.69 (C, Ar), 144.73 , (C=N), 163.26 , (C=O). ν_{max} (solid)/(cm^{-1}) 3337 (md), 1611 (st), 1566 (st), 1492 (st), 1319 (st), 1208 (md), 1147 (st), 1121 (st), 1073 (st), 828 (st). MS *m/z* (**API-ES**): found 503 $(M^{35}C+NH_4)^+$ (100%), 505 $(M^{37}C+NH_4)$ (35%). HRMS *m/z* (**API-ES**): found 503.0894 $(M+NH_4)^+$, calculated for $C_{21}H_{20}ClN_6O_5S$ 503.0904; found 486.0626 $(M+H)^+$, calculated for $C_{21}H_{16}N_5O_5S$ 486.0639.

***3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid (thiophen-2-ylmethyl)amide* (191a$_{14}$)**. This was obtained as a yellow solid (0.090 g, 0.169 mmol, 59%) from **190j** (0.107 g, 0.133 mmol) and 2-nitrophenylhydrazine (0.055 g, 0.366 mmol) in a similar manner as described for preparation of **191a$_2$**, mp 270 °C (dec). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 4.16 (2H, d, *J* 6.4 Hz, CH_2), 6.86-6.87 (2H, m), 7.08 (1H, d, *J* 8.4 Hz, H-7), 7.20 (1H, t, *J* 8.0 Hz, ArH), 7.35 (1H, d, *J* 4.0 Hz, ArH), 7.74 (1H, d, *J* 8.0 Hz, ArH), 7.81 (1H, t, *J* 8.0 Hz, ArH), 8.00 (1H, s, ArH), 8.16-8.24 (3H, m, 2 x CH, Ar & $HNSO_2$), 11.56 (1H, s, HNCO), 14.23 (1H, s, HNN). ^{13}C NMR (100 MHz , DMSO-d$_6$) δ 42.08 (CH_2), 111.66 (CH, Ar), 116.68 (CH, Ar), 118.98 (CH, Ar), 121.47 (C, Ar), 122.89 (CH, Ar), 126.37 (CH, Ar), 126.57 (CH, Ar), 126.76 (CH, Ar), 127.35 (CH, Ar), 129.68 (CH, Ar), 132.63 (C, Ar), 133.97 (C, Ar), 134.98 (C, Ar), 137.29 (CH, Ar), 139.67 (C, Ar), 141.31 (C, Ar), 144.80 (C=N), 163.29 (C=O). ν_{max} (solid)/(cm^{-1}) 1684 (md), 1615 (md), 1559 (md), 1492 (st), 1157 (st), 789 (st). MS *m/z* (**API-ES**): found 475 $(M+NH_4)^+$ (100%). HRMS *m/z* (**API-ES**): found 475.0852 $(M+NH_4)^+$, calculated for $C_{19}H_{19}N_6O_5S_2$ 475.0858.

***3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid (pyridin-4-ylmethyl)amide* (191a$_{18}$)**. This was obtained as a yellow solid (0.012 g, 0.256 mmol, 38%) from **190n** (0.022 g, 0.069 mmol) and 2-nitrophenylhydrazine (0.011 g, 0.076 mmol) in a similar manner as described for preparation of **191a$_2$**, mp 279-281 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 4.30 (2H, d, *J* 6.0 Hz, CH_2), 7.10 (1H, d, *J* 7.2 Hz, H-7), 7.22 (1H, t, *J* 8.4 Hz, ArH), 7.75 (1H, d, J 7.2Hz, H-6), 7.79-783 (3H, m, ArH), 8.02 (1H, s, H-4), 8.22-8.25 (2H, m, ArH), 8.53 (1H, t, *J* 6.0 Hz, $HNSO_2$), 8.75 (1H, d, *J* 5.8 Hz, 2 x CH, Ar), 11.65 (1H, s, HNCO), 14.24 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 45.68 (CH_2), 111.79 (CH, Ar), 116.73 (CH, Ar), 118.95 (CH, Ar), 121.59 (2 x C, Ar), 123.01 (CH, Ar), 124.58 (CH, Ar), 124.98 (2 x CH, Ar), 126.62 (CH, Ar), 129.71 (C, Ar), 132.51 (CH, Ar), 134.54 (C,

Ar), 137.29 (C, Ar), 138.95 (CH, Ar), 139.64 (C, Ar), 144.75 (C, Ar), 145.02 , (C=N), 163.29 , (C=O). ν_{max} (solid)/(cm^{-1}) 3283 (md), 1681 (md), 1613 (md), 1484 (st), 1334 (st), 1112 (st), 782 (st). MS *m/z* (**API-ES**): found 453 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 453.0978 $(M+H)^+$, calculated for $C_{20}H_{17}N_6O_5S$ 453.0981.

***3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid (2-dimethylaminoethyl)amide* (191a$_6$).** This was obtained as a yellow solid (0.018 g, 0.041 mmol, 24%) from **190b** (0.050 g, 0.168 mmol) and 2-nitrophenylhydrazine (0.028 g, 0.185 mmol) in a similar manner as described for preparation of **191a$_2$**, mp 180 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.74 (3H, s, NCH_3), 2.75 (3H, s, NCH_3), 3.07-3.13 (4H, m, $C\underline{H}_2C\underline{H}_2$), 7.14 (1H, d, *J* 8.3 Hz, H-7), 7.20-7.24 (1H, m, ArH), 7.78 (1H, dd, *J* 1.8, 8.3 Hz, H-6), 7.79-7.83 (1H, m, ArH), 7.96-7.98 (1H, m, $HNSO_2$), 8.06 (1H, d, *J* 1.8 Hz, H-4), 8.22-8.26 (2H, m, ArH), 11.66 (1H, s, HNCO), 14.25 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.97 ($NHCH_2\underline{C}H_2$), 45.84 ($N(C\underline{H}_3)_2$), 58.81 ($NH\underline{C}H_2CH_2$), 111.14 (CH, Ar), 116.54 (CH, Ar), 118.87 (CH, Ar), 121.46 (C, Ar), 122.91 (CH, Ar), 126.67 (CH, Ar), 129.63 (CH, Ar), 132.72 (C, Ar), 133.95 (C, Ar), 134.98 (C, Ar), 137.34 (CH, Ar), 139.69 (C, Ar), 144.73 (C=N), 163.34 (C=O). ν_{max} (solid)/(cm^{-1}) 3275 (md), 1676 (md), 1603 (md), 1476 (st), 1051 (st). MS *m/z* (**API-ES**): found 433 $(M+H)^+$. HRMS *m/z* (**API-ES**): found 433.1293 $(M+H)^+$, calculated for $C_{18}H_{21}N_6O_5S$ 433.1294.

***1-Methyl-3-[(2-nitro-phenyl)-hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide* (191a$_3$).** This was obtained as a yellow solid (0.022 g, 0,054 mmol, 36%) from **190s** (0.040 g, 0.149 mmol) and 2-nitrophenylhydrazine (0.025 g, 0.164 mol) in a similar manner as described for preparation of **191a$_2$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ δ 2.62 (6H, s, $N(CH_3)_2$), 3.32 (3H, s, NCH_3), 7.20-7.25 (1H, m, ArH), 7.39 (1H, d, *J* 8.4 Hz, H-7), 7.78 (1H, dd, *J* 1.6, 8.4 Hz, ArH), 7.79-7.83 (1H, m, ArH), 7.99 (1H, d, *J* 1.6 Hz, H-4), 8.23 (1H, dd, *J* 1.6, 8.4 Hz, H-6), 8.33 (1H, d, *J* 8.4 Hz, ArH), 14.25 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.67 (CH_3), 38.34 (2 x CH_3), 111.89 (CH, Ar), 116.87 (CH, Ar), 119.58 (CH, Ar), 121.71 (C, Ar), 122.72 (CH, Ar), 126.56 (CH, Ar), 128.93 (C, Ar), 130.73 (CH, Ar), 132.25 (C, Ar), 133.96 (C, Ar), 137.47 (CH, Ar), 139.60 (C, Ar), 145.37 (C=N), 163.28 (C=O). ν_{max} (solid)/(cm^{-1}) 1624 (st) , 1546 (st), 1478 (st), 1357 (st), 1116(st). MS *m/z* (**API-ES**): found 404 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 404.1034 $(M+H)^+$, calculated for $C_{17}H_{18}N_5O_5S$ 404.1029.

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid (furan-2-ylmethyl)amide (191a$_{13}$)

A mixture of **190i** (0.040 g, 0.114 mmol) 2-nitrophenylhydrazine (0.021 g, 0.126 mmol) and HCl (aq 4 M, 2 drops) in ethanol (3 mL) was heated in the CEM microwave at 120 °C for 15 min. After cooling to room temperature, pure product **191a$_{13}$** was collected as an orange

precipitate by filtration and dried in vacuo (0.048 g, 0.099 mmol, 86%), mp 245 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.99 (2H, d, *J* 6.0 Hz, CH_2), 6.17 (1H, dd, *J* 0.8, 3.2 Hz, ArH), 6.28 (1H, dd, *J* 2.0, 3.2 Hz, ArH), 7.07 (1H, d, *J* 8.2 Hz, H-7), 7.19-7.24 (1H, m, ArH), 7.46 (1H, dd, *J* 0.8, 1.6 Hz, ArH), 7.71 (1H, dd, *J* 1.6, 8.2 Hz, H-6), 7.80-7.84 (1H, m, ArH), 7.98 (1H, d, *J* 1.6 Hz, H-4), 8.08 (1H, t, *J* 6.0 Hz, $HNSO_2$), 8.22-8.26 (2H, m, ArH), 11.55 (1H, s, NHCO), 14.24 (1H, s, NNH) . ^{13}C NMR (100 MHz, DMSO-d_6) δ 40.01 (CH_2), 108.69 (CH, Ar), 111.07 (CH, Ar), 111.55 (CH, Ar), 116.66 (CH, Ar), 118.93 (CH, Ar), 121.39 (C, Ar), 122.87 (CH, Ar), 126.57 (CH, Ar), 129.62 (CH, Ar), 132.66 (C, Ar), 133.95 (C, Ar), 135.03 (C, Ar), 137.28 (CH, Ar), 139.67 (C, Ar), 143.20 (CH, Ar), 144.71 (C, Ar), 151.18 (C=N), 163.29 (C=O). ν_{max} (solid)/(cm^{-1}) 3620 (md), 3178 (md), 1686 (md), 1614 (md), 1558 (md), 1489 (st), 1340 (md), 1147 (st). MS *m/z* (**API-ES**): found 459 $(M+NH_4)^+$ (100%). HRMS *m/z* (**API-ES**): found 459.1082 $(M+NH_4)^+$, calculated for $C_{19}H_{19}N_6O_6S$ 459.1087.

3-[(3-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonicamide **(191a$_{32}$)**. This was obtained as a yellow solid (0.041 g, 0.113 mmol, 49%) from **190a** (0.053 g, 0.234 mmol) and 3-nitrophenylhydrazine (0.039 g, 0.257 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.05 (1H, d, *J* 8.0 Hz, H-7), 7.30 (2H, s, NH_2), 7.64 (1H, t, *J* 8.2 Hz, ArH), 7.72 (1H, dd, *J* 1.6, 8.0 Hz, H-6), 8.87 (1H, d, *J* 8.2 Hz, ArH), 8.93 (1H, d, *J* 8.2 Hz, ArH), 8.00 (1H, s, H-4), 8.61-8.37 (1H, m, ArH), 11.44 (1H, s, HNCO), 12.76 (1H, s, HNN). ^{13}C NMR (400 MHz, DMSO-d_6) δ 109.32 (CH, Ar), 111.07 (CH, Ar), 117.36 (CH, Ar), 117.95 (CH, Ar), 121.64 (C, Ar), 121.76 (CH, Ar), 127.61 (CH, Ar), 129.29 (C, Ar), 131.46 (CH, Ar), 138.63 (C, Ar), 143.43 (C, Ar), 144.60 (C, Ar), 149.46 (C=N), 163.51 (C=O). ν_{max} (solid)/(cm^{-1}) 3445 (md), 3321 (md), 1675 (st), 1623(md), 1563 (st), 1373 (st), 1142 (st). MS *m/z* (**API-ES**): found 362 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 362.0557 $(M+H)^+$, calculated for $C_{14}H_{12}N_5O_5S$ 362.0559.

3-[43-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonicamide **(191a$_{34}$)**. This was obtained as a yellow solid (0.049 g, 0.135 mmol, 59%) from **190a** (0.052 g, 0.230 mmol) and 4-nitrophenylhydrazine (0.038 g, 0.253 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.06 (1H, d, *J* 8.4 Hz, H-7), 7.30 (2H, s, NH_2), 7.68 (1H, d, *J* 9.2 Hz, 2 x CH, ArH), 7.72 (1H, dd, *J* 1.8, 8.4 Hz, H-6), 7.75 (1H, d, *J* 1.8 Hz, ArH), 8.24 (1H, d, *J* 9.2 Hz, ArH), 8.00 (1H, s, H-4), 11.49 (1H, s, HNCO), 12.83 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d_6) δ 111.28 (CH, Ar), 115.10 (2 x CH, Ar), 117.75 (CH, Ar), 121.48 (C, Ar), 126.40 (2 x CH, Ar), 128.22 (CH, Ar), 131.17 (C, Ar), 138.77 (C, Ar), 142.67 (C, Ar), 143.92 (C, Ar), 148.75 (C=N), 163.49 (C=O). ν_{max} (solid)/(cm^{-1}) 3452 (md), 3319 (md), 1686 (st), 1645 (st), 1521 (md), 1388 (st), 1196 (st). MS *m/z* (**API-ES**): found 362 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 362.0558 $(M+H)^+$, calculated for $C_{14}H_{12}N_5O_5S$ 362.0559.

***3-[(3-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide* (191a$_{33}$).** This was obtained as a yellow solid (0.046 g, 0.950 mmol, 68%) from **190p** (0.049 g, 0.140 mmol) and 3-nitrophenylhydrazine (0.023 g, 0.154 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 3.97 (2H, d, *J* 6.6 Hz, CH_2), 7.03 (1H, d, *J* 8.4 Hz, H-7), 7.23 (2H, d, *J* 8.4 Hz, 2 x CH, ArH), 7.27 (2H, d, *J* 8.4 Hz, 2 x CH, ArH), 7.59-7.66 (2H, m, ArH), 7.83 (1H, s, H-4), 7.86 (1H, d, *J* 8.0 Hz, ArH), 7.96 (1H, d, *J* 8.4 Hz, ArH), 8.15 (1H, t, *J* 6.6 Hz, $HNSO_2$), 8.37 (1H, s, ArH), 11.46 (1H, s, HNCO), 12.77 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 46.13 (CH_2), 109.47 (CH, Ar), 111.23 (CH, Ar), 118.00 (CH, Ar), 118.06 (CH, Ar), 121.65 (CH, Ar) 121.95 (C, Ar), 128.65 (CH, Ar), 128.75 (2 x CH, Ar), 129.03 (C, Ar), 130.16 (2 x CH, Ar), 131.46 (C, Ar), 132.37 (C, Ar), 134.82 (C, Ar), 137.34 (C, Ar), 143.86 (C, Ar), 144.59 (C, Ar), 149.25 (C=N), 163.46 (C=O). ν_{max} (solid)/(cm^{-1}) 1623 (st), 1531 (st), 1480 (st), 1321 (st), 1147 (st), 1145 (st), 1068 (st). MS *m/z* (**API-ES**): found 486 ($M^{35}C$+H)$^+$ (100%), 488 ($M^{37}C$+H)$^+$ (35%). HRMS *m/z* (**API-ES**): found 486.0639 (M+H)$^+$, calculated for $C_{21}H_{16}N_5O_5S$ 486.0639.

***3-[(4-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide* (191a$_{35}$).** This was obtained as a yellow solid (0.043 g, 0.099 mmmol, 74%) from **190p** (0.047 g, 0.134 mmol) and 4-nitrophenylhydrazine (0.022 g, 0.147 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp > °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 3.98 (2H, d, *J* 6.2 Hz, CH_2), 7.04 (1H, d, *J* 8.0 Hz, H-7), 7.27 (2H, d, *J* 8.4 Hz, 2 x CH, ArH), 7.27 (2H, d, *J* 8.4 Hz, 2 x CH, ArH), 7.67-7.71 (3H, m, ArH), 7.84 (1H, s, *J* 1.6 Hz, H-4), 8.14 (1H, t, *J* 6.2 Hz, $HNSO_2$), 8.25 (2H, d, *J* 9.2 Hz, 2 x CH, ArH), 11.51 (1H, s, HNCO), 12.82 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 46.15 (CH_2), 111.45 (CH, Ar), 115.13 (2 x CH, Ar), 118.50 (CH, Ar), 121.24 (C, Ar), 126.33 (2 x CH, Ar), 128.76 (2 x CH, Ar), 129.16 (CH, Ar), 130.16 (2 x CH, Ar), 130.85 (C, Ar), 132.38 (C, Ar), 135.01 (C, Ar), 137.31 (C, Ar), 142.68 (C, Ar), 144.31 (C, Ar), 148.67 (C=N), 163.43 (C=O). ν_{max} (solid)/(cm^{-1}) 1612 (st), 1566 (st), 1434 (st), 1356 (st), 1112 (st), 1156 (st), 781 (st). MS *m/z* (**API-ES**): found 486 ($M^{35}C$+H)$^+$ (100%), 488 ($M^{37}C$+H)$^+$ (35%). HRMS *m/z* (**API-ES**): found 486.0638 (M+H)$^+$, calculated for $C_{21}H_{16}N_5O_5S$ 486.0639.

***3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid benzylamide* (191a$_{36}$).** This was obtained as a yellow solid (0.104 g, 0.230 mmol, 74%) from **190q** (0.098 g, 0.310 mmol) and 2-nitrophenylhydrazine (0.052 g, 0.341 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 286 °C (dec). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 3.98 (2H, d, *J* 6.6 Hz, CH_2), 7.09 (1H, d, *J* 8.4 Hz, H-7), 7.17-7.28 (6H, m, ArH), 7.75 (1H, dd, *J* 1.6, 8.4 Hz, H-6), 7.82 (1H, t, *J* 7.8 Hz, ArH), 8.00 (1H, s, H-4), 8.09 (1H, t, *J* 6.6 Hz, $HNSO_2$), 8.22-8.26 (2H, m, ArH), 11.58 (1H, s, HNCO), 14.25 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 46.85 (CH_2), 111.66 (CH, Ar), 116.79 (CH, Ar) 118.95 (CH, Ar),

121.45 (C, Ar), 122.89 (CH, Ar), 126.58 (CH, Ar), 127.80 (2 x CH, Ar), 128.31 (2 x CH, Ar), 128.90 (2 x CH, Ar), 129.64 (CH, Ar), 132.64 (C, Ar), 133.96 (C, Ar), 135.09 (C, Ar), 137.29 (2 x CH, Ar), 138.27 (C, Ar), 139.67 (C, Ar), 144.73 (C=N), 163.29 (C=O). ν_{max} (solid)/(cm^{-1}) 1623 (st), 1536 (st), 1442 (st), 1326 (st), 1131 (md), 845 (st). MS *m/z* (**API-ES**): found 452 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 452.1046 $(M+H)^+$, calculated for $C_{21}H_{18}N_5O_5S$ 452.1029.

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide **(191a$_8$)**. This was obtained as a yellow solid (0.034 g, 0.074 mmol50%) from **190d** (0.047 g, 0.173 mmol) and 2-nitrophenylhydrazine (0.029 g, 0.192 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 285 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (6H, d, *J* 6.4 Hz, $CH(\underline{CH_3})_2$), 7.10 (1H, d, *J* 8.3 Hz, H-7), 7.19-7.23 (1H, m, ArH), 7.49 (1H, d, *J* 7.2 Hz, $HNSO_2$), 7.75 (1H, dd, *J* 1.7, 8.3 Hz, H-6), 7.80-7.83 (1H, m, ArH), 8.02 (1H, d, *J* 1.7 Hz, H-4), 8.21-8.24 (2H, m, ArH), 11.55 (1H, s, NHCO), 14.23 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.90 ($CH(\underline{C}H_3)_2$), 45.93 ($\underline{C}H(CH_3)_2$), 111.71 (CH, Ar), 116.68 (CH, Ar), 118.76 (CH, Ar), 121.42 (C, Ar), 122.89 (CH, Ar), 126.59 (C, Ar), 129.74 (CH, Ar), 132.75 (CH, Ar), 133.97 (CH, Ar), 136.28 (C, Ar), 137.31 (CH, Ar), 139.67 (C, Ar), 144.63 (C=N), 163.29 (C=O). ν_{max} (solid)/(cm^{-1}) 3295 (md), 1692 (md), 1614 (md), 1553 (md), 1490 (st), 1340 (md), 1314 (md), 1153 (st), 1138 (st), 1072 (md). MS *m/z* (**API-ES**): found 421 $(M+NH_4)^+$ (100%), 404 $(M+H)^+$ (80%). HRMS *m/z* (**API-ES**): found 421.1285 $(M+NH_4)^+$, calculated for $C_{17}H_{21}N_6O_5S$ 421.1294; found 404.1016 $(M+H)^+$, calculatd for $C_{17}H_{18}N_5O_5S$ 404.1029.

3-[(2-Nitrophenyl)-hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid propylamide **(191a$_7$)**. This was obtained as a yellow solid (0.034 g, 0.084 mmol, 56%) from **190c** (0.02 g, 0.156 mmol) and 2-nitrophenylhydrazine (0.026 g, 0.172 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 277-279 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78 (3H, t, *J* 7.2 Hz, $CH_2CH_2C\underline{H_3}$), 1.37 (2H, sex, *J* 7.3 Hz, $CH_2C\underline{H_2}CH_3$), 2.67 (2H, q, *J* 6.4 Hz, $C\underline{H_2}CH_2CH_3$), 7.10 (1H, d, *J* 8.0 Hz, H-7), 7.21 (1H, t, *J* 7.6 Hz, ArH), 7.48 (1H, t, *J* 5.6 Hz, $HNSO_2$), 7.73 (1H, d, *J* 7.6 Hz, ArH), 7.81 (1H, t, *J* 7.6 Hz, ArH), 8.01 (1H, s, H-4), 8.21-8.24 (2H, m, ArH), 7.92 (1H, dd, *J* 2.0, 8.4, Hz, H-6), 11.55 (1H, s, NHCO) 14.23 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.87 ($CH_2CH_2\underline{C}H_3$), 23.80 ($CH_2\underline{C}H_2CH_3$), 45.07 ($\underline{C}H_2CH_2CH_3$), 111.68 (CH, Ar), 116.67 (CH, Ar), 118.84 (CH, Ar), 121.50 (C, Ar), 122.88 (CH, Ar), 126.57 (CH, Ar), 129.61 (CH, Ar), 132.71 (C, Ar), 133.96 (C, Ar), 134.98 (C, Ar), 137.29 (CH, Ar), 139.67 (C, Ar), 144.72 (C=N), 163.29 (C=O). ν_{max} (solid)/(cm^{-1}) 3363 (md), 3296 (md), 3252 (md), 1693 (md), 1680 (md), 1613 (md), 1570 (st), 1491 (st), 1321 (md), 1296 (md), 1143 (st), 1072 (md). MS *m/z* (**API-ES**): found 421 $(M+NH_4)^+$ (100%), 404 $(M+H)^+$ (60%). MS *m/z* (**API-ES**): found 404 $(M+H)^+$ (100%).

HRMS *m/z* (**API-ES**): found 421.1287, $(M+NH_4)^+$, calculated for $C_{17}H_{21}N_6O_5S$ 421.1294, found 404.1014 $(M+H)^+$, calculated for $C_{17}H_{18}N_5O_5S$ 404.1029.

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid (2-methoxyethyl)amide (**191a$_9$**). This was obtained as a yellow solid (0.035 g, 0.835 mmol, 49%) from **190e** (0.050 g, 0.176 mmol) and 2-nitrophenylhydrazine in a similar manner as described for preparation of **191a$_{13}$**, mp 252-254 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.89 (2H, q, *J* 6.0 Hz, $C\underline{H}_2CH_2OCH_3$), 3.14 (3H, s, OCH_3), 7.10 (1H, d, *J* 8.4 Hz, H-7), 7.19-7.23 (1H, m, ArH), 7.65 (1H, t, *J* 6.0 Hz, $HNSO_2$), 7.74 (1H, dd, *J* 2.0, 8.0 Hz, H-6), 7.80-7.84 (1H, m, ArH), 8.03 (1H, d, *J* 2.0 Hz, H-4), 8.21-8.25 (2H, m, ArH), 11.57 (1H, s, NHCO), 14.24 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.90 ($CH_2\underline{C}H_2OCH_3$), 58.58 ($OCH_3$), 71.23 ($\underline{C}H_2CH_2OCH_3$), 111.16 (CH, Ar), 116.64 (CH, Ar), 118.90 (CH, Ar), 121.45 (C, Ar), 122.84 (CH, Ar), 126.55 (CH, Ar), 129.60 (CH, Ar), 132.70 (C, Ar), 133.92 (C, Ar), 135.03 (C, Ar), 137.25 (CH, Ar), 139.66 (C, Ar), 144.75 (C=N), 163.28 (C=O). ν_{max} (solid)/(cm^{-1}) 3287 (md), 1691 (md), 1612 (md), 1554 (md), 1491 (st), 1315 (md), 1073 (st). MS *m/z* (**API-ES**): found 420 $(M+H)^+$ (100%), 437 $(M+NH_4)^+$ (40%). HRMS *m/z* (**API-ES**): found 437.1243 $(M+NH_4)$, calculted for $C_{17}H_{21}N_6O_6S$ 437.1243; found 420.0979 $(M+H)^+$, calculated for $C_{17}H_{18}N_5O_6S$ 420.0978.

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid (tetrahydrofuran-2-ylmethyl)amide (**191a$_{12}$**). This was obtained as a yellow solid (0.057 g, 0.126 mmol, 63%) yield from **190h** (0.065 g, 0.200 mmol) and 2-nitrophenylhydrazine (0.034 g, 0.227 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 285-287 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.47-1.55 (1H, m), 1.73-1.75 (2H, m), 1.79-1.85 (1H, m), 2.75 (2H, t, *J* 5.6 Hz), 3.54 (1H, q, *J* 7.6 Hz), 3.65 (1H, q, *J* 7.2 Hz), 3.79 (1H, quint, *J* 5.6 Hz), 7.10 (1H, d, *J* 8.2 Hz, H-7), 7.21 (1H, t, *J* 7.6 Hz, ArH), 7.66 (1H, t, *J* 7.6 Hz, ArH), 7.74 (1H, d, *J* 8.2 Hz, H-6), 7.80-7.83 (1H, m, ArH), 8.03 (1H, s, H-4), 8.22-8.25 (2H, m, ArH), 11.56 (1H, s, NHCO), 14.24 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.76 (CH_2), 29.10 (CH_2), 47.28 (CH_2), 67.97 (CH_2), 77.68 (CH), 111.63 (CH, Ar), 116.67 (CH, Ar), 118.92 (CH, Ar), 121.45 (C, Ar), 122.87 (CH, Ar), 126.57 (CH, Ar), 129.62 (CH, Ar), 132.73 (C, Ar), 133.96 (C, Ar), 135.09 (C, Ar), 137.28 (CH, Ar), 139.67 (C, Ar), 144.74 (C=N), 163.29 (C=O). ν_{max} (solid)/(cm^{-1}) 3244 (md), 3154 (md), 1690 (md), 1612 (md), 1510 (md), 1488 (st), 1330 (md), 1149 (st). MS *m/z* (**API-ES**): found 446 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 463.1398 $(M+NH_4)^+$, calculated for $C_{19}H_{23}N_6O_6S$ 463.1400; found: 446.1138 $(M+H)^+$, calculated for $C_{19}H_{20}N_5O_6S$ 446.1134.

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid sec-butylamide (**191a$_{10}$**). This was obtained as a yellow solid (0.026 g, 0.062 mmol, 50%) from **190f** (0.035 g, 0.124 mmol) and 2-nitrophenylhydrazine (0.020 g, 0.136 mmol) in a similar manner as

described for preparation of **191a$_{13}$**, mp 289-291 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 0.70 (3H, t, *J* 7.6 Hz, NCHCH$_2$C<u>H$_3$</u>), 0.86 (3H, d, *J* 6.4 Hz, NCHC<u>H$_3$</u>), 1.29 (2H, quint, *J* 7.2 Hz, NCHC<u>H$_2$</u>CH$_3$), 3.04 (1H, quint, *J* 7.2 Hz, NC<u>H</u>CH$_2$CH$_3$), 7.10 (1H, d, *J* 8.4 Hz, H-7), 7.21 (1H, t, *J* 8.4 Hz, ArH), 7.43 (1H, d, *J* 7.2 Hz, HNSO$_2$), 7.75 (1H, dd, *J* 1.6, 8.4 Hz, H-6), 7.82 (1H, t, *J* 8.4 Hz, ArH), 8.02 (1H, s, H-4), 8.23-8.24 (1H, s, ArH), 11.55 (1H, s, NHCO), 14.24 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 10.75 (CH$_3$), 21.31 (CH$_3$), 30.28 (CH), 51.36 (CH$_2$), 111.68 (CH, Ar), 116.69 (CH, Ar), 118.73 (CH, Ar), 121.35 (C, Ar), 122.88 (CH, Ar), 126.58 (CH, Ar), 129.44 (CH, Ar), 132.76 (C, Ar), 133.98 (C, Ar), 136.59 (C, Ar), 137.30 (CH, Ar), 139.69 (C, Ar), 144.57 (C=N), 163.29 (C=O). ν_{max} (solid)/(cm^{-1}) 3285 (md), 1691 (md), 1614 (md), 1552 (md), 1489 (st), 1313 (md), 1151 (st), 1136 (st), 1071 (md). MS *m/z* (**API-ES**): found 418 (M+H)$^+$ (100%), 435 (M+NH$_4$)$^+$ (60%). HRMS *m/z* (**API-ES**): found 435.1448 (M+NH$_4$)$^+$, calculated for $C_{17}H_{21}N_6O_6S$ 435.1451; found 418.1181, calculated for $C_{18}H_{20}N_5O_5S$ 418.1185.

1-Ethyl-3-[(2-nitro-phenyl)-hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide **(191a$_4$)**. This was obtained as a yellow solid (0.021 g, 0.0503 mmol, 62%) from **190t** (0.023 g, 0.081 mmol) and 2-nitrophenylhydrazine (0.012 g, 0.0815 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 289-291 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 1.23 (3H, t, *J* 7.2 Hz, CH$_2$C<u>H$_3$</u>), 2.63 (6H, s, N(CH$_3$)$_2$), 3.88 (2H, q, *J* 7.2 Hz, C<u>H$_2$</u>CH$_3$), 7.22 (1H, t, *J* 7.9 Hz, H-7), 7.76-7.82 (2H, m, ArH), 7.99 (1H, s, H-4), 7.76-7.82 (1H, m, ArH), 8.22 (1H, d, *J* 7.4 Hz, H-6), 8.32 (1H, d, *J* 7.9 Hz, ArH), 14.24 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 13.43 (CH$_2$<u>C</u>H$_3$), 35.11 (<u>C</u>H$_2$CH$_3$), 38.03 (2 x CH$_3$), 110.72 (CH, Ar), 117.04 (CH, Ar), 119.43 (CH, Ar), 121.41 (C, Ar), 123.14 (CH, Ar), 126.54 (CH, Ar), 129.49 (C, Ar), 130.54 (CH, Ar), 131.61 (C, Ar), 134.09 (C, Ar), 137.31 (CH, Ar), 139.50 (C, Ar), 145.34 (C=N), 161.21 (C=O). ν_{max} (solid)/(cm^{-1}) 1690 (md), 1607 (md), 1566 (md), 1499 (md), 1336 (st), 1181 (md), 1142 (st), 1112 (ms). MS *m/z* (**API-ES**): found 418 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 435.1448 (M+NH$_4$)$^+$, calculated for $C_{17}H_{21}N_6O_6S$ 435.1451; found 418.1180 (M+H)$^+$, calculated for $C_{18}H_{20}N_5O_5S$: 418.1185.

5-(Morpholine-4-sulfonyl)-3-[(2-nitrophenyl)hydrazono]-1,3-dihydro-indol-2-one **(191a$_{11}$)**. This was obtained as a yellow solid (0.040 g, 0.089 mmol, 56%) from **190g** (0.050 g, 0.159 mmol) and 2-nitrophenylhydrazine (0.024 g, 0.156 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 2.87 (4H, s, 2 x CH$_2$), 3.62 (4H, s, 2 x CH$_2$), 7.17 (1H, d, *J* 8.4 Hz, H-7), 7.21 (1H, t, *J* 8.1 Hz, ArH), 7.68 (1H, d, *J* 8.4 Hz, H-6), 7.80 (1H, t, *J* 8.1 Hz, ArH), 7.94 (1H, s, H-4), 8.22 (1H, d, *J* 8.1 Hz, ArH), 8.31 (1H, d, *J* 8.1 Hz, ArH), 11.65 (1H, s, NHCO), 14.25 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 46.64 (2 x CH$_2$), 65.96 (2 x CH$_2$), 111.93 (CH, Ar), 116.94 (CH, Ar), 119.76 (CH, Ar), 121.77 (C, Ar), 122.99 (CH, Ar), 126.50 (CH, Ar), 128.54 (C, Ar),

130.73 (CH, Ar), 132.36 (C, Ar), 133.98 (C, Ar), 137.23 (CH, Ar), 139.56 (C, Ar), 145.57 (C=N), 163.21 (C=O). ν_{max} (solid)/(cm^{-1}) 3267 (md), 1702 (st), 1615 (st), 1572 (st), 1490 (st0, 1344 (st0, 1292 (st), 1149 (st), 1076 (st), 940 (st). MS *m/z* (**API-ES**): found 499 $(M+NH_4)^+$ (100%). HRMS *m/z* (**API-ES**): found 449.1240 $(M+NH_4)^+$, calculated for $C_{18}H_{21}N_6O_6S$ 449.1243; found: 432.0974 $(M+H)^+$, calculated for $C_{18}H_{17}N_5O_6S$: 432.0978.

1-Benzyl-3-[(2-nitro-phenyl)-hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide **(191a$_5$)**. This was obtained as a yellow solid (0.010 g, 0.020 mmol, 21%) from **190u** (0.034 g, 0.098 mmol) and 2-nitrophenylhydrazine (0.015 g, 0.098 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 149-151 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.69 (6H, s, N(C<u>H$_3$</u>)$_2$), 5.19 (2H, s, CH_2), 7.26-7.33 (3H, m, Ar), 7.37 (2H, t, *J* 7.6 Hz, ArH), 7.48 (2H, d, *J* 7.6 Hz, ArH), 7.77 (1H, d, *J* 8.0 Hz, H-6), 7.85 (1H, t, *J* 7.6 Hz, H-5'), 8.01 (1H, s, H-4), 8.30 (1H, d, *J* 8.8 Hz, CH, Ar), 8.41 (1H, d, *J* 8.8 Hz, CH, Ar), 14.26 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 38.23 (2 x CH_3), 44.67 (CH_2), 111.72 (CH, Ar), 116.73 (CH, Ar), 117.80 (C, Ar), 119.67 (CH, Ar), 121.82 (C, Ar), 122.59 (CH, Ar), 124.83 (CH, Ar), 126.48 (CH, Ar), 127.62 (2 x CH, Ar), 128.81 (2 x CH, Ar), 128.86 (C, Ar), 130.17 (CH, Ar), 132.22 (C, Ar), 133.84 (C, Ar), 137.62 (CH, Ar), 139.60 (C, Ar), 145.21 (C=N), 163.32 (C=O). ν_{max} (solid)/(cm^{-1}) 1693 (md), 1608 (md), 1568 (md), 1496 (st), 1330 (st), 1153 (st). MS *m/z* (**API-ES**): found 480 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 480.1289 $(M+H)^+$, calculated for $C_{23}H_{22}N_5O_5S$ 480.1342.

2-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazino]benzoic acid **(191a$_{23}$)**. This was obtained as a yellow solid (0.038 g, 0.086 mmol, 68%) from **190d** (0.034 g, 0.126 mmol) and 2-carboxylphenylhydrazine (0.026 g, 0.139 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 270 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.4 Hz, CH(C<u>H$_3$</u>)$_2$), 7.06 (1H, d, *J* 8.0 Hz, H-7), 7.11 (1H, t, *J* 7.6 Hz, ArH), 7.45 (1H, d, *J* 7.6 Hz, $HNSO_2$), 7.63-7.70 (2H, m, ArH), 8.93-7.97 (2H, m, ArH), 8.04 (1H, d, *J* 8.0 Hz, ArH), 11.31 (1H, s, NHCO), 14.45 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.91 (CH(<u>C</u>H$_3$)$_2$), 45.91 (<u>C</u>H(CH$_3$)$_2$), 111.19 (CH, Ar), 114.76 (CH, Ar), 114.83, 117.91 (CH, Ar), 122.21 (C, Ar), 122.73 (CH, Ar), 128.32 (C, Ar), 129.49 (C, Ar), 132.03 (CH, Ar), 135.18 (CH, Ar), 135.83 (C, Ar) , 143.81 (C, Ar) , 145.10 (C=N), 162.89 (C=O), 168.90 (C=O). ν_{max} (solid)/(cm^{-1}) 3277 (st), 1689 (md), 1558 (md), 1498 (md), 1154 (st). MS *m/z* (**API-ES**): found 403 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 403.1066 $(M+H)^+$, calculated for $C_{18}H_{19}N_4O_5S$ 403.1076.

2-[N'-(2-Oxo-5-sulfamoyl-1,2-dihydro-indol-3-ylidene)hydrazino]benzoic acid **(191a$_{24}$)**. This was obtained as a yellow solid (0.025 g, 0.053 mmol, 30%) from **190a** (0.047 g, 0.207 mmol) and 2-carboxylphenylhydrazine (0.039 g, .0207 mmol) in a similar manner as

described for preparation of **191a$_{13}$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.05 (1H, d, *J* 8.1 Hz, H-7), 7.11 (1H, t, *J* 8.0 Hz, ArH), 7.27 (2H, s, NH_2), 7.65 (1H, t, *J* 8.4 Hz, ArH), 7.71 (1H, dd, *J* 1.8, 8.1 Hz, H-6), 7.89 (1H, dd, *J* 1.6, 8.0 Hz, ArH), 8.02-8.04 (2H, m, ArH), 11.35 (1H, s, NHCO), 14.26 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 110.95 (CH, Ar), 114.74 (CH, Ar), 117.36 (CH, Ar) 122.02 (CH, Ar), 122.69 (C, Ar), 127.74 (CH, Ar), 129.64 (C, Ar), 132.05 (CH, Ar), 135.20 (CH, Ar), 138.44 (C, Ar), 143.55 (C, Ar), 145.15 (C=N), 162.94 (C=O), 168.90 (C=O). ν_{max} (solid)/(cm^{-1}) 3307 (st), 3252 (st), 1691 (st), 1565 (st), 1496 (st), 1321 (st), 1147 (st). MS *m/z* (**API-ES**): found 359 (M-H)$^-$. HRMS *m/z* (**API-ES**): found 359.0452 (M-H)$^-$, calculated for $C_{15}H_{12}N_4O_5S$ 359.0450.

2-[N'-[5-(4-Chloro-benzylsulfamoyl)-2-oxo-1,2-dihydro-indol-3-ylidene]hydrazine]benzoic acid **(191a$_{25}$)**. This was obtained as a yellow solid (0.029 g, 0.055 mmol, 66%) from **190p** (0.029 mmol) and 2-carboxylphenylhydrazine (0.015 g, 0.083 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 290-292 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 3.97 (2H, d, *J* 6.0 Hz, CH_2), 7.03 (1H, d, *J* 8.0 Hz, H-7), 7.10-7.14 (1H, m, ArH), 7.23 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.27 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.64-7.67 (2H, m, $HNSO_2$ & ArH), 7.87 (1H, d, *J* 1.6 Hz, H-4), 7.95 (1H, dd, *J* 1.6, 8.0 Hz, H-6), 8.04-8.10 (2H, m, ArH), 11.31 (1H, s, NHCO), 14.26 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 46.13 (CH_2), 111.11 , (CH, Ar), 114.78 , (CH, Ar), 114.86 , (C, Ar), 118.07 , (CH, Ar), 122.22 , (CH, Ar), 122.76 , (C, Ar), 128.16 , (CH, Ar), 128.77 (2 x CH, Ar), 129.35 , (C, Ar), 130.16 (2 x CH, Ar), 132.04 , (C, Ar), 132.35 , (CH, Ar), 134.62 , (CH, Ar), 135.20 , (C, Ar), 137.41 , (C, Ar), 143.94 , (C, Ar), 145.13 (C=N), 162.88 (C=O), 168.91 (C=O). ν_{max} (solid)/(cm^{-1}) 3236 (md), 1682 (md), 1500 (md), 1145 (st). MS *m/z* (**API-ES**): found 483 (M ^{35}Cl-H)$^-$ (100%), 485 (M^{37}Cl-H)$^-$ (35%). HRMS *m/z* (**API-ES**): found: 483.0517(M-H)$^-$, calculated for $C_{22}H_{17}ClN_4O_5S$ 483.0530.

3-(Phenylhydrazono)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide **(191a$_{21}$)**. This was obtained as a yellow solid (0.031 g, 0.086 mmol, 48%) from **190d** (0.048 g, 0179 mmol) and phenylhydrazine (0.019 g, 0.179 mmol) in a similar manner as described for preparation of **181a$_{13}$**, mp 267-269 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 0.93 (6H, d, *J* 6.8 Hz, CH(<u>CH$_3$</u>)$_2$), 3.18-3.23 (1H, m, <u>C</u>H(CH$_3$)$_2$), 7.05-7.09 (2H, m, H-7 & ArH), 7.36-7.40 (2H, m, ArH), 7.45 (3H, m, 2 x ArH & $HNSO_2$), 7.63-7.70 (2H, m, ArH), 7.66 (1H, d, *J* 1.7, 8.1 Hz, H-6), 7.92 (1H, d, *J* 1.7 Hz, H-4), 11.40 (1H, s, NHCO), 12.73 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 23.90 , (CH(<u>CH$_3$</u>)$_2$), 45.90 , (<u>C</u>H(CH$_3$)$_2$), 111.18 , (CH, Ar), 115.26 , (2 x CH, Ar), 117.29 , (CH, Ar), 122.26 , (CH, Ar), 124.26 , (C, Ar), 127.05 , (C, Ar), 127.61 , (CH, Ar), 130.23 , (2 x CH, Ar), 130.73 , (C, Ar), 135.85 , (C, Ar), 142.92 , (C=N), 163.86 (C=O). ν_{max} (solid)/(cm^{-1}) 3288 (md), 3164 (md), 1680 (st), 1551 (st), 1168 (md), 1135 (md), 1120 (md). MS *m/z* (**API-ES**): found 359 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 359.1169 (M+H)$^+$, calculated for $C_{17}H_{19}N_4O_3S$ 359.1178.

3-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzoic acid (**191a$_{26}$**). This was obtained as a yellow solid (0.062 g, 0.154 mmol, 57%) from **190d** (0.075 g, 0.297 mmol) and 3-carboxylphenylhydrazine (0.047 g, 0.307 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 275 °C (dec). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 0.93 (6H, d, *J* 6.8 Hz, CH(C<u>H$_3$</u>)$_2$), 3.21 (1H, sept, *J* 6.8 Hz, C<u>H</u>(CH$_3$)$_2$), 7.05 (1H, d, *J* 8.4 Hz, H-7), 7.47-7.51 (2H, m, HNSO$_2$ & ArH), 7.45 (1H, d, *J* 8.0 Hz, ArH), 7.67-7.70 (3H, m, ArH), 7.92 (1H, d, *J* 2.0 Hz, H-4), 8.05-8.06 (1H, m, ArH), 11.43 (1H, s, NHCO), 12.76 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 23.88 (CH(<u>C</u>H$_3$)$_2$), 45.88 (<u>C</u>H(CH$_3$)$_2$), 111.18 (CH, Ar), 115.36 (CH, Ar), 117.44 (CH, Ar), 119.75 (CH, Ar), 122.20 (CH, Ar), 124.74 (C, Ar), 127.90 (CH, Ar), 127.97 (C, Ar), 130.43 (C, Ar), 132.79 (CH, Ar), 135.89 (C, Ar), 143.24 (C, Ar), 143.30 (C=N), 163.76 (C=O), 167.72 (C=O). ν_{max} (solid)/(cm^{-1}) 3285 (md), 1682 (st), 1553 (st), 1464 (md), 1295 (md), 1168 (st), 1121 (md). MS *m/z* (**API-ES**): found 403 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 403.1095 (M+H)$^+$, calculated for $C_{18}H_{19}N_4O_5S$ 403.1076.

3-[N'-(2-Oxo-5-sulfamoyl-1,2-dihydro-indol-3-ylidene)hydrazino]benzoic acid (**191a$_{27}$**). This was obtained as a yellow solid (0.048 g, 0.134 mmol, 58%) from **190a** (0.052 g, 0.253 mmol) and 3-carboxylphenylhydrazine (0.038 g, 0.253 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.05 (1H, d, *J* 8.4 Hz, H-7), 7.29 (2H, s, NH$_2$), 7.49 (1H, t, *J* 8.0 Hz, ArH), 7.63 (1H, d, *J* 7.6 Hz, ArH), 7.67-7.71 (2H, m, ArH), 7.98 (1H, d, *J* 2.0 Hz, H-4), 8.06 (1H, s, ArH), 10.40 (1H, s, HNCO), 12.76 (1H, s, HNN), 13.12 (1H, s, CO$_2$H). ^{13}C NMR (400 MHz, DMSO-d$_6$) δ 110.96 (CH, Ar), 115.33 (CH, Ar), 116.93 (CH, Ar), 119.78 (CH, Ar), 121.98 (CH, Ar), 124.69 (C, Ar), 127.06 (C, Ar), 128.00 (CH, Ar), 130.45 (C, Ar), 132.80 (CH, Ar), 138.53 (C, Ar), 142.94 (C, Ar), 143.36 (C=N), 163.78 (C=O), 167.71 (C=O). ν_{max} (solid)/(cm^{-1}) 3343 (st), 3224 (st), 1685 (st), 1557 (st), 1496 (st), 1339 (st), 1153 (st). MS *m/z* (**API-ES**): found 359 (M-H)$^-$ (100%). HRMS *m/z* (**API-ES**): found 359.0469 (M-H)$^-$, calculated for $C_{15}H_{11}N_4O_5S$ 359.0450.

4-[N'-(2-Oxo-5-sulfamoyl-1,2-dihydro-indol-3-ylidene)hydrazino]benzoic acid (**191a$_{30}$**). This was obtained as a yellow solid (0.044 g, 0.122 mmol, 53%) from **190a** (0.053 g, 0.234 mmol) and 4-carboxylphenylhydrazine (0.039 g, 0.257 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.05 (1H, d, *J* 8.4 Hz, H-7), 7.28 (2H, s, NH$_2$), 7.55 (2H, d, *J* 8.6 Hz, 2 x CH, ArH), 7.72 (1H, d, *J* 1.6, 8.4 Hz, ArH), (2H, d, *J* 8.6 Hz, 2 x CH, ArH), 8.00 (1H, d, *J* 1.6 Hz, H-4), 10.44 (1H, s, HNCO), 12.74 (1H, s, CO$_2$H), 12.76 (1H, s, HNN). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 111.12 (CH, Ar), 114.71 (2 x CH, Ar), 117.27 (CH, Ar), 121.75 (C, Ar), 125.69 (C, Ar), 127.49 (C, Ar), 129.19 (CH, Ar), 131.79 (2 x CH, Ar), 138.64 (C, Ar), 143.29 (C, Ar),

146.62 (C=N), 163.75 (C=O), 167.56 (C=O). ν_{max} (solid)/(cm^{-1}) 3315 (st), 3221 (st), 1686 (st), 1543 (st), 1496 (st), 1337 (st), 1112 (st). MS *m/z* (**API-ES**): found 359 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 359.0467 $(M-H)^-$, calculated for $C_{15}H_{11}N_4O_5S$ 359.0450.

***3-[N'-[5-(4-Chloro-benzylsulfamoyl)-2-oxo-1,2-dihydro-indol-3-ylidene]hydrazine]benzoic acid* (191a$_{28}$)**. This was obtained as a yellow solid (0.043 g, 0.088 mmol, 55%) from **190p** (0.056 g, 0.160 mmol) and 3-carboxylphenylhydrazine (0.026 g, 0.176 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.96 (2H, d, *J* 6.4 Hz, CH_2), 7.03 (1H, d, *J* 8.0 Hz, H-7), 7.23 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.28 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.49 (1H, d, *J* 7.8 Hz, ArH), 7.64-7.65 (2H, m, ArH), 7.70-7.72 (1H, m, ArH), 7.81 (1H, s, H-4), 7.06 (1H, s, ArH), 8.16 (1H, t, *J* 6.4 Hz, $HNSO_2$), 11.44 (1H, s, NHCO), 12.76 (1H, s, NNH), 13.14 (1H, bs, CO_2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.12 (CH_2), 111.12 (CH, Ar), 115.46 (CH, Ar), 117.59 (CH, Ar), 119.77 (CH, Ar), 122.19 (C, Ar), 124.76 (CH, Ar), 127.77 (C, Ar), 128.14 (CH, Ar), 128.75 (2 x CH, Ar), 130.45 (2 x CH, Ar), 130.46 (CH, Ar), 132.65 (C, Ar), 132.82 (C, Ar), 134.68 (C, Ar), 137.36 (C, Ar), 143.36 (C, Ar), 143.77 (C=N), 163.74 (C=O), 167.72 (C=O). ν_{max} (solid)/(cm^{-1}) 3209 (st), 1661 (st), 1523 (md), 1147 (st). MS *m/z* (**API-ES**): found 483 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 483.0436 $(M-H)^-$, calculated for $C_{22}H_{16}N_4O_5SCl$ 483.0530.

***4-[N'-[5-(4-Chloro-benzylsulfamoyl)-2-oxo-1,2-dihydro-indol-3-ylidene]hydrazine]benzoic acid* (191a$_{31}$)**. This was obtained as a yellow solid (0.051 g, 0.105 mmol, 77%) from **190p** (0.048 g, 0.137 mmol) and 4-carboxylphenylhydrazine (0.022 g, 0.150 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.97 (2H, d, *J* 6.3 Hz, CH_2), 7.03 (1H, d, *J* 7.8 Hz, H-7), 7.23 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.28 (2H, d, *J* 8.6 Hz, 2 x CH, Ar), 7.57 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.66 (1H, dd, *J* 1.6, 7.8 Hz, H-6), 7.84 (1H, d, *J* 1.6 Hz, H-4), 7.94 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 8.13 (1H, t, *J* 6.3 Hz, $HNSO_2$), 11.44 (1H, s, NHCO), 12.76 (1H, s, NNH), 13.14 (1H, bs, CO_2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.12, 11.29, 114.79, 117.96, 121.94, 125.73, 128.76, 128.92, 130.15, 131.79, 131.82, 132.34, 134.82, 137.34, 143.71, 146.61 (C=N), 163.68 (C=O), 167.57 (C=O). ν_{max} (solid)/(cm^{-1}) 3221 (st), 1685 (st), 1545 (st), 1143 (st). MS *m/z* (**API-ES**): found 483 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 483.0437 $(M-H)^-$, calculated for $C_{22}H_{16}N_4O_5SCl$ 483.0530.

***4-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzoic acid* (191a$_{29}$)**. This was obtained as a yellow solid (0.046 g, 0.114 mmol, 75%) from **190d** (0.039 g, 0.152 mmol) and 4-carboxylphenylhydrazine (0.023 g, 0.152 mmol) in a similar manner as described for preparation of **191a$_{13}$**, mp 290 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.0 Hz, $CH(\underline{CH_3})_2$), 7.07 (1H, d, *J* 8.0 Hz, H-7), 7.48 (1H, d, *J* 7.2 Hz, $HNSO_2$),

7.56 (1H, d, *J* 8.0 Hz, 2 x CH, Ar), 7.70 (1H, d, *J* 8.0 Hz, H-6), 7.94 (2H, d, *J* 8.0 Hz, 2 x CH, Ar), 7.95 (1H, m, H-4), 11.47 (1H, s, NHCO), 12.74 (1H, bs, CO_2H). 12.77 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.91 (CH($\underline{CH_3}$)$_2$), 45.90 ($\underline{CH}$(CH_3)$_2$), 111.35 (CH, Ar), 114.77 (2 x CH, Ar), 117.83 (CH, Ar), 121.95 (C, Ar), 125.72 (C, Ar), 128.35 (C, Ar), 129.08 (CH, Ar), 131.78 (2 x CH, Ar), 136.02 (C, Ar), 143.59 (C, Ar), 146.57 (C, Ar), 163.70 (C=N), 167.56 (C=O). ν_{max} (solid)/(cm^{-1}) 3263 (md), 1695 (st), 1535 (st), 1443 (st), 1145 (st), 1112 (md). MS *m/z* (**API-ES**): found 401 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 401.0848 $(M-H)^-$, calculated for $C_{18}H_{17}N_4O_5S$ 401.0920.

***3-(Naphthyl-hydrazono)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide* (191a$_{22}$).** This was obtained as a red solid (45%) from **190d** and 1-naphthylhydrazine in a similar manner as described for preparation of **191a$_{13}$**, mp 215-217 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 0.95 (6H, d, *J* 6.8 Hz, CH($\underline{CH_3}$)$_2$), 3.13-3.27 (1H, m, $\underline{CH}$(CH_3)$_2$), 7.13 (1H, d, *J* 8.4 Hz, H-7), 7.49 (1H, d, *J* 7.2 Hz, $NHSO_2$), 7.58 (1H, d, *J* 7.9 Hz, ArH), 7.61 (1H, d, *J* 8.4 Hz, ArH), 7.64-7.72 (3H, m, 2 x ArH & H-6), 7.87 (1H, d, *J* 7.9 Hz, ArH), 7.89 (1H, d, *J* 7.9 Hz, ArH), 7.99-8.01 (2H, m, 2 x ArH & H-4), 11.62 (1H, s, NHCO), 13.78 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.92 (CH($\underline{CH_3}$)$_2$), 45.93 ($\underline{CH}$(CH_3)$_2$), 109.73 (CH, Ar), 111.52 (CH, Ar), 117.64 (CH, Ar), 119.75 (CH, Ar), 121.85 (C, Ar), 122.39 (C, Ar), 124.01 (CH, Ar), 127.20 (CH, Ar), 127.36 (CH, Ar), 127.96 (CH, Ar), 129.20 (C, Ar), 129.50 (CH, Ar), 134.45 (C, Ar), 136.10 (C, Ar), 137.11 (C, Ar), 143.12 (C=N), 164.67 (C=O). ν_{max} (solid)/(cm^{-1}) 3259 (md), 3167 (md), 1675 (md), 1561 (st), 1321 (md), 1195 (st), 1155 (st), 1070 (md), 1006 (md), 825 (st), 782 (st), 766 (st). MS *m/z* (**API-ES**): found 409 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 409.1324 $(M+H)^+$, calculated for $C_{21}H_{21}N_4O_3S$ 409.1334.

***2-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzoic acid pentafluorophenyl ester* (192a)[394]**

Anhydrous pyridine (0.362 g, 4.59 mmol) and pentafluorophenyltrifluoro acetate (1.28 g, 4.59 mmol) were added to a solution of **191a$_{23}$** (1.23 g, 3.06 mmol) in anhydrous in DMF (15 ml) at room temperature under Ar. The reaction mixture was stirred for 1 h at room temperature. Pentafluorophenyltrifluoro acetate (0.325 g, 1.16 mmol) and anhydrous pyridine (0.204 g, 2.58 mmol) were added. The reaction mixture was stirred for 30 min and poured into water (20 ml). The product was extracted with ethyl acetate (3 x 40 ml), dried over Na_2SO_4 and the solvent removed under reduced pressure to provide a yellow solid. The pure compound **192a** was obtained after trituration with a solution ethyl acetate/hexane (3:7, 40 ml) as a yellow solid (1.40 g, 3.38 mmol, 78%), mp 190-192 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.8 Hz, CH($\underline{CH_3}$)$_2$), 3.19-3.24 (1H, m, $\underline{CH}$(CH_3)$_2$), 7.07 (1H, d, *J* 8.4 Hz, H-7), 7.25 (1H, t, *J* 7.6 Hz, ArH), 7.48 (1H, d, *J* 6.8 Hz, $HNSO_2$), 7.72 (1H, d, *J* 8.4 Hz, ArH), 7.86 (1H, t, *J* 7.6 Hz, ArH), 8.00 (1H, s, ArH), 8.18 (1H, d, *J* 8.4 Hz, ArH), 8.23

(1H, d, *J* 7.6 Hz, ArH), 11.44 (1H, s, NHCO), 13.97 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.95 (CH($\underline{CH_3}$)$_2$), 46.01 ($\underline{CH}$(CH$_3$)$_2$), 111.32 (CH, Ar), 114.43 (CH, Ar), 114.85 (CH, Ar), 118,03 (CH, Ar), 122.24 (C, Ar), 122.88 (CH, Ar), 128.25 (C, Ar), 129.86 (C, Ar), 131.91 (CH, Ar), 134.67-135.54 (m) (CF), 135.18 (CH, Ar), 136.06 (C, Ar), 135.56-136.96 (m) (2 x CF), 141.02-142.12 (m) (2 x CF), 143.76 (C, Ar) , 144,96 (C=N), 162.54 (C=O), 170.03 (C=O). ν_{max} (solid)/(cm^{-1}) 1672 (st), 1598 (st), 1467 (st), 1234 (md), 1154 (st). MS *m/z* (**API-ES**): found 569 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 569.0915 (M+H)$^+$, calculated for $C_{24}H_{18}F_5N_4O_5S$ 569.0918.

***3-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzoic acid pentafluorophenyl ester* (192b)**. This was obtained as a yellow solid (1.22 g, 2,086 mmol, 80%) from **191a$_{26}$** (1.05 g, 2.911 mmol) and pentafluorophenyltrifluoro acetate (0.877 g, 3.134 mmol) in a similar manner as described for preparation of **192a**. ^{1}H NMR (400 MHz, DMSO-d_6) 0.93 (6H, d, *J* 6.6 Hz, CH($\underline{CH_3}$)$_2$), 3.16-3.21 (1H, m, $\underline{CH}$(CH$_3$)$_2$), 7.07 (1H, d, *J* 8.0 Hz, H-7), 7.47 (1H, t, *J* 6.6 Hz, NHSO$_2$), 7.63-7.70 (2H, m, ArH), 7.85 (1H, d, *J* 8.0 Hz, ArH), 7.93-7.97 (2H, m, ArH), 8.29 (1H, s, ArH), 11.45 (1H, s, NHCO), 12.81 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.79 (CH($\underline{CH_3}$)$_2$), 45.79 ($\underline{CH}$(CH$_3$)$_2$), 110.97 (CH, Ar), 114.87 (CH, Ar), 117.32 (CH, Ar), 120.23 (CH, Ar), 121.92 (CH, Ar), 124.32 (C, Ar), 127.90 (CH, Ar), 128.02 (C, Ar), 130.67 (C, Ar), 132.74 (CH, Ar), 134.32-135.12 (m) (CF), 136.01 (C, Ar), 135.23-136.58 (m) (2 x CF), 141.99-142.67 (m) (2 x CF), 143.34 (C, Ar), 144.12 (C=N), 163.34 (C=O), 168.23 (C=O). ν_{max} (solid)/(cm^{-1}) 1656 (st), 1591 (st), 1487 (md), 1204 (md), 1032 (md). MS *m/z* (**API-ES**): found 569 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 569.0917 (M+H)$^+$, calculated for $C_{24}H_{18}F_5N_4O_5S$ 569.0918

N-Furan-2-ylmethyl-2-[N'-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3ylidene)hydrazino]benzamide (193a)[394]

Anhydrous pyridine (0.030 g, 0.379 mmol) and furfurylamine (0.038 g, 0.390 mmol) were added to a solution of **192a** (0.155 g, 0.264 mmol) anhydrous in acetonitrile (20 ml) at room temperature under Ar. The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure to provide a yellow solid. The pure compound **193a** was obtained after trituration with acetone (7 ml) as a brown solid, mp 253-255 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (6H, d, *J* 6.8 Hz, CH($\underline{CH_3}$)$_2$), 3.19-3.22 (1H, m, $\underline{CH}$(CH$_3$)$_2$), 4.49 (2H, d, *J* 5.4 Hz, CH$_2$), 6.31 (1H, d, *J* 3.2 Hz, ArH), 6.39, 6.4 (1H, m, ArH), 7.05 (1H, d, *J* 8.2 Hz, H-7), 7.12 (1H, t, *J* 7.6 Hz, ArH), 7.45 (1H, d, *J* 7.2 Hz, N$\underline{H}$SO$_2$), 7.56-7.60 (2H, m, ArH), 7.68 (1H, d, *J* 8.2 Hz, H-6), 7.80 (1H, d, *J* 7.6 Hz, ArH), 7.95 (1H. s, H-4), 8.01 (1H, d, *J* 7.6 Hz, ArH), 9.17 (1H, d, *J* 5.4 Hz, CON$\underline{H}$CH$_2$), 11.29 (1H, s, N$\underline{H}$CO), 14.10 (1H, s, NN$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.92 (CH($\underline{CH_3}$)$_2$), 36.63 (CH$_2$), 45.89 ($\underline{CH}$(CH$_3$)$_2$), 107.68 (CH, Ar), 111.05 (CH, Ar), 111.24 (CH, Ar), 115.22 (CH, Ar),

117.65 (CH, Ar), 118.60 (CH, Ar), 122.41 (C, Ar), 122.80 (C, Ar), 128.70 (C, Ar), 129.03 (CH, Ar), 133.42 (CH, Ar), 135.70 (C, Ar), 136.50 (C, Ar), 142.77 (CH, Ar), 143.59 (CH, Ar), 143.79 (C=N), 152.79 (CH, Ar), 162.74 (C=O), 167.83 (C=O). ν_{max} (solid)/(cm^{-1}) 3271 (md), 1678 (md), 1616 (md), 1509 (st), 1330 (md), 1185 (md), 1157 (st). MS *m/z* (**API-ES**): found 482 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 482.1489 $(M+H)^+$, calculated for $C_{23}H_{24}N_5O_5S$ 482.1498.

2-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzamide **(193b)**. This was obtained as a yellow solid (0.040 g, 0.099 mmol, 38%) from **192a** (0.155 g, 0.264 mmol) and ammonia (2M solution in ethanol) (0.198 ml, 0.396 mmol) in a similar manner as described for preparation of **193a**, mp 291-293 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.4 Hz, $CH(C\underline{H}_3)_2$), 3.89-3.23 (1H, m, $C\underline{H}(CH_3)_2$), 7.03 (1H, d, *J* 8.0 Hz, H-7), 7.07 (1H, t, *J* 7.6 Hz, ArH), 7.44 (1H, d, *J* 7.2 Hz, $N\underline{H}SO_2$), 7.56 (1H, t, *J* 7.6 Hz, ArH), 7.58 (1H, bs, CONH), 7.66 (1H, dd, *J* 1.4, 8.0 Hz, H-6), 7.78 (1H, d, *J* 7.6 Hz, ArH), 7.94 (1H, d, J 1.4 Hz, H-4), 8.00 (1H, d, *J* 7.6 Hz, ArH), 8.16 (1H, bs, CONH), 11.13 (1H, s, $N\underline{H}CO$), 14.42 (1H, s, $NN\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.92 ($CH(\underline{C}H_3)_2$), 45.88 ($\underline{C}H(CH_3)_2$), 110.99 (CH, Ar), 115.03 (CH, Ar), 117.59 (C, Ar), 118.59 (CH, Ar), 122.48 (C, Ar), 122.69 (CH, Ar), 127.93 (CH, Ar), 128.48 (C, Ar), 129.42 (CH, Ar), 133.36 (CH, Ar), 135.64 (C, Ar), 143.52 (C, Ar), 144.01 (C=N), 162.71 (C=O), 170.29 (C=O). ν_{max} (solid)/(cm^{-1}). ν_{max} (solid)/(cm^{-1}) 3172 (md), 1612 (md), 1557 (md), 1498 (st), 1386 (md), 1297 (md), 117 (st). MS *m/z* (**API-ES**): found 402 $(M+H)^+$ (100%), found 385 $(M-NH_2)^+$ (30%). HRMS *m/z* (**API-ES**): found 402.1227 $(M+H)^+$, calculated for $C_{18}H_{20}N_5O_4S$ 402.1236.

N-(2-Dimethylaminoethyl)-2-[N'-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzamide **(193c)**. This was obtained as a yellow solid (0.042 g, 0.088 mmol, 37%) from **192a** (0.138 g, 0.235 mmol) and *N*,*N*-dimethylethylenediamine (0.031 g, 0.352 mmol) in a similar manner as described for preparation of **193a**, mp 250-252 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.4 Hz, $CH(C\underline{H}_3)_2$), 2.17 (6H, s, $N(C\underline{H}_3)_2$), 2.40 (2H, t, *J* 6.8 Hz, $CONHCH_2C\underline{H}_2$), 3.16-3.24 (1H, m, $C\underline{H}(CH_3)_2$), 7.04 (1H, d, *J* 8.2 Hz, H-7), 7.11 (1H, t, *J* 7.8 Hz, ArH), 7.44 (1H, d, *J* 7.2 Hz, $N\underline{H}SO_2$), 7.55 (1H, t, *J* 7.8 Hz, ArH), 7.66 (1H, d, *J* 8.2 Hz, H-6), 7.73 (1H, d, *J* 7.8 Hz, ArH), 7.94 (1H, d, J 1.6 Hz, H-4), 8.00 (1H, d, *J* 7.8 Hz, ArH), 8.59 (1H, bs, CONH), 11.24 (1H, s, $N\underline{H}CO$), 14.25 (1H, s, $NN\underline{H}$). ^{13}C NMR (400 MHz, DMSO-d_6) δ 23.92 ($CH(\underline{C}H_3)_2$), 37.97 ($CONHCH_2\underline{C}H_2$), 45.91 ($N(C\underline{H}_3)_2$), 45.88 ($\underline{C}H(CH_3)_2$), 58.63 ($CONH\underline{C}H_2CH_2$), 111.01 (CH, Ar), 115.12 (CH, Ar), 117.56 (C, Ar), 119.30 (CH, Ar), 122.48 (C, Ar), 122.79 (CH, Ar), 127.90 (CH, Ar), 128.48 (C, Ar), 128.87 (CH, Ar), 133.12 (CH, Ar), 135.64 (C, Ar), 143.49 (C, Ar), 143.61 (C=N), 162.73 (C=O), 167.77 (C=O). ν_{max} (solid)/(cm^{-1}) 3284 (st), 1679 (st), 1632 (st), 1514 (st), 1456 (md), 1329 (md), 1176 (st), 1156 (st). MS *m/z* (**API-ES**): found 473

$(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 473.1975 $(M+H)^+$, calculated for $C_{22}H_{29}N_6O_4S$ 473.1971.

2-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazino]-N-(2-methoxyethyl)benzamide **(193d).** This was obtained as a yellow solid (0.046 g, 0.100 mmol, 49%) from **192a** (0.123 g, 0.205 mmol) and 2-methoxyethylamine (0.023 g, 0.314 mmol) in a similar manner as described for preparation of **193a**, mp 287-289 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.4 Hz, $CH(C\underline{H}_3)_2$), 3.18-3.23 (1H, m, $C\underline{H}(CH_3)_2$), 3.26 (3H, s, OCH_3), 3.42-3.47 (4H, m, $COC\underline{H}_2C\underline{H}_2$), 7.04 (1H, d, *J* 8.4 Hz, H-7), 7.11 (1H, t, *J* 7.8 Hz, ArH), 7.44 (1H, d, *J* 6.8 Hz, $N\underline{H}SO_2$), 7.56 (1H, t, *J* 7.8 Hz, ArH), 7.66 (1H, d, *J* 8.4 Hz, H-6), 7.75 (1H, d, *J* 7.8 Hz, ArH), 7.94 (1H, s, H-4), 7.99 (1H, d, *J* 7.8 Hz, ArH), 8.72 (1H, bs, CONH), 11.28 (1H, s, $N\underline{H}CO$), 14.28 (1H, s, $NN\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.91 ($CH(\underline{C}H_3)_2$), 39.59 ($CONHCH_2\underline{C}H_2$), 45.89 ($\underline{C}H(CH_3)_2$), 58.62 ($CONH\underline{C}H_2CH_2$), 111.01 (CH, Ar), 70.96 (OCH_3), 111.04 (CH, Ar), 115.14 (CH, Ar), 117.61 (CH, Ar), 119.04 (C, Ar), 122.43 (C, Ar), 122.79 (CH, Ar), 127.96 (CH, Ar), 128.56 (C, Ar), 128.91 (CH, Ar), 133.20 (CH, Ar), 135.67 (C, Ar), 143.54 (C, Ar), 143.59 (C=N), 162.75 (C=O), 167.98 (C=O). ν_{max} (solid)/(cm^{-1}) 3274 (st), 1679 (st), 1634 (md), 1511 (st), 1455 (md), 1511 (st), 1328 (md), 1180 (md), 1154 (st), 1116 (st). MS *m/z* (**API-ES**): found 460 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 460.1652 $(M+H)^+$, calculated for $C_{21}H_{26}N_5O_5S$ 460.1655.

N-Benzyl-2-[N'-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzamide **(193e)**. This was obtained as a yellow solid (0.068 g, 0.084 mmol, 84%) from **192a** (0.096 g, 0.163 mmol) and benzylamine (0.026 g, 0.244 mmol) in a similar manner as described for preparation of **193a**, mp 275-277 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.8 Hz, $CH(C\underline{H}_3)_2$), 3.19-3.22 (1H, m, $C\underline{H}(CH_3)_2$), 4.50 (2H, d, *J* 5.2 Hz, CH_2), 7.04 (1H, d, *J* 8.2 Hz, H-7), 7.13 (1H, t, *J* 7.6 Hz, ArH), 7.21-7.24 (1H, m, ArH), 7.30-7.34 (4H, m, ArH), 7.45 (1H, d, *J* 6.8 Hz, $N\underline{H}SO_2$), 7.58 (1H, t, *J* 7.6 Hz, ArH), 7.68 (1H, d, *J* 8.2 Hz, H-6), 7.84 (1H, d, *J* 7.6 Hz, ArH), 7.95 (1H, s, H-4), 8.02 (1H, d, *J* 7.6 Hz, ArH), 9.24 (1H, bs, CONH), 11.28 (1H, s, $N\underline{H}CO$), 14.32 (1H, s, $NN\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.91 ($CH(\underline{C}H_3)_2$), 43.09 (CH_2), 45.89 ($\underline{C}H(CH_3)_2$), 111.05 (CH, Ar), 115.23 (CH, Ar), 117.63 (CH, Ar), 118.87 (C, Ar), 122.42 (C, Ar), 122.86 (CH, Ar), 127.51 (CH, Ar), 127.88 (2 x CH, Ar), 127.99 (CH, Ar), 128.65 (C, Ar), 128.92 (CH, Ar), 129.03 (2 x CH, Ar), 133.35 (CH, Ar), 135.68 (C, Ar), 140.02 (C, Ar), 143.57 (C, Ar), 143.75 (C=N), 162.75 (C=O), 167.89 (C=O). ν_{max} (solid)/(cm^{-1}) 3267 (md), 1681 (st), 1635 (st), 1500 (st), 1319 (st0, 1182 (st), 11.59 (st). MS *m/z* (**API-ES**): found 492 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 492.1698 $(M+H)^+$, calculated for $C_{25}H_{26}N_5O_4S$ 492.1706.

2-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]-N-pyridin-3-ylmethylbenzamide **(193f)**. This was obtained as a yellow solid (0.066 g, 0.130 mmol, 37%)

from **192a** (0.139 g, 0.236 mmol) and 3-(aminomethyl)pyridine in a similar manner as described for preparation of **193a**, mp 210 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 7.0 Hz, CH(C$\underline{H}_3$)$_2$), 4.53 (1H, d, *J* 5.8 Hz, CH_2), 7.07 (1H, d, *J* 8.6 Hz, H-7), 7.23 (1H, t, *J* 5.9 Hz, $NHSO_2$), 7.39-7.42 (1H, m, ArH), 7.46 (1H, d, *J* 7.6 Hz, ArH), 7.61 (1H, d, *J* 7.6 Hz, ArH), 7.68 (1H, dd, *J* 1.4, 8.6 Hz, H-6), 7.78-7.83 (3H, m, ArH), 7.99 (1H, s, H-4), 8.14 (1H, d, *J* 7.6 Hz, ArH), 8.43 (1H, m, ArH), 8.61 (1H, s, ArH), 9.29 (1H, t, *J* 5.8 Hz, CONH), 11.35 (1H, s, N$\underline{H}$CO), 14.25 (1H, s, NN$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.91 (CH(C$\underline{H}_3$)$_2$), 40.92 (CH_2), 45.90 (C$\underline{H}$(CH$_3$)$_2$), 111.48 (CH, Ar), 115.24 (CH, Ar), 117.66 (CH, Ar), 118.65 (C, Ar), 122.39 (C, Ar), 122.85 (CH, Ar), 124.46 (CH, Ar), 128.01 (CH, Ar), 128.73 (C, Ar), 128.96 (CH, Ar), 133.47 (CH, Ar), 135.70 (C, Ar), 136.44 (CH, Ar), 143.59 (C, Ar), 143.79 (C=N), 148.39 (CH, Ar), 149.00 (CH, Ar), 163.77 (C=O), 168.09 (C=O). ν_{max} (solid)/(cm^{-1}) 3274 (st), 1697 (md), 1502 (st), 1453 (md), 1287 (ms), 1155 (st). MS *m/z* (**API-ES**): found 493 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 493.1649 $(M+H)^+$, calculated for $C_{24}H_{25}N_6O_4S$ 493.1658.

2-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]-N-(2-morpholin-4-yl-ethyl)benzamide **(193g)**. This was obtained as a yellow solid (0.050 g, 0.097 mmol, 67%) from **192a** (0.165 g, 0.281 mmol) and 2-morpholin-4-yl-ethylamine (0.054 g, 0.412 mmol) in a similar manner as described for preparation of **193a**, mp 220 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.8 Hz, CH(C$\underline{H}_3$)$_2$), 3.18-3.23 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 3.58 (4H, bs, 2 x CH_2) 7.04 (1H, d, *J* 8.4 Hz, H-7), 7.13 (1H, t, *J* 7.6 Hz, ArH), 7.45 (1H, d, *J* 7.2 Hz, N$\underline{H}$SO$_2$), 7.57 (1H, t, *J* 7.6 Hz, ArH), 7.67 (1H, d, *J* 8.4 Hz, H-6), 7.84 (1H, d, *J* 7.6 Hz, ArH), 7.94 (1H, s, H-4), 8.02 (1H, d, *J* 7.6 Hz, ArH), 8.68 (1H, bs, CONH), 11.29 (1H, s, N$\underline{H}$CO), 14.23 (1H, s, NN$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.83 (CH(C$\underline{H}_3$)$_2$), 37.35 (CH_2), 45.79 (C$\underline{H}$(CH$_3$)$_2$), 53.76 (2 x CH_2), 58.09 (CH_2), 66.83 (2 x CH_2), 111.16 (CH, Ar), 113.64 (CH, Ar), 117.45 (CH, Ar), 117.87 (CH, Ar), 122.23 (C, Ar), 122.82 (CH, Ar), 127.54 (C, Ar), 128.03 (CH, Ar), 130.39 (CH, Ar), 136.04 (C, Ar), 136.46 (C, Ar), 143.23 (C, Ar), 143.34 (C=N), 163.84 (C=O), 167.58 (C=O). ν_{max} (solid)/(cm^{-1}) 3315 (md), 1692 (md), 1629 (md), 1500 (st), 1300 (md), 1156 (st), 1116 (st). MS *m/z* (**API-ES**): found 515 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 515.2070 $(M+H)^+$, calculated for $C_{24}H_{31}N_6O_5S$ 515.2077.

2-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]-N-methylbenzamide **(193h)**. This was obtained as a yellow solid (0.056 g, 0.134 mmol, 69%) from **192a** (0.114 g, 0.194 mmol) and methylamine (40% solution in water) (0.025 ml, 0.291 mmol) in a similar manner as described for preparation of **193a**, mp 185 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.8 Hz, CH(C$\underline{H}_3$)$_2$), 2.79 (3H, d, *J* 4.4 Hz, HNC$\underline{H}_3$), 3.19-3.25 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 7.04 (1H, d, *J* 8.1 Hz, H-7), 7.11 (1H, t, *J* 8.0 Hz, ArH), 7.44 (1H, d, *J* 6.8 Hz, N$\underline{H}$SO$_2$), 7.56 (1H, t, *J* 8.0 Hz, ArH), 7.67 (1H, d, *J* 1.7, 8.1 Hz, H-6), 7.73

(1H, d, *J* 8.0 Hz, ArH), 7.94 (1H, d, *J* 1.7 Hz, H-4), 8.00 (1H, d, *J* 8.0 Hz, ArH), 8.63 (1H, bq, *J* 4.4 Hz, HNCH$_3$), 11.31 (1H, s, NHCO), 14.32 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 23.83 (CH(CH$_3$)$_2$), 26.87 (CH$_3$), 45.81 (CH(CH$_3$)$_2$), 111.09 (CH, Ar), 113.53 (CH, Ar), 117.46 (CH, Ar), 117.73 (CH, Ar), 122.21 (C, Ar), 122.85 (CH, Ar), 127.63 (C, Ar), 127.81 (CH, Ar), 130.45 (CH, Ar), 136.03 (C, Ar), 136.56 (C, Ar), 143.15 (C, Ar), 143.18 (C=N), 163.65 (C=O), 166.79 (C=O). ν_{max} (solid)/(cm^{-1}) 1687 (md), 1615 (md), 1499 (st), 1298 (md), 1155 (st). MS *m/z* (**API-ES**): found 416 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 416.1385 (M+H)$^+$, calculated for $C_{19}H_{22}N_5O_4S$ 416.1392.

N-Ethyl-2-[N'-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzamide **(193i)**.This was obtained as a yellow solid (0.089 g, 0.207 mmol, 75%) from **192a** (0.162 g, 0.275 mmol) and methylamine (70% solution in water) (0.033 ml, 0.413 mmol) in a similar manner as described for preparation of **193a**, mp 290 °C (dec). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 0.93 (6H, d, *J* 6.4 Hz, CH(CH$_3$)$_2$), 1.13 (3H, t, *J* 7.0 Hz, HNCH$_2$CH$_3$), 3.19-3.25 (1H, m, CH(CH$_3$)$_2$), 7.04 (1H, d, *J* 8.2 Hz, H-7), 7.11 (1H, t, *J* 7.4 Hz, ArH), 7.44 (1H, d, *J* 7.2 Hz, NHSO$_2$), 7.55 (1H, t, *J* 7.8 Hz, ArH), 7.67 (1H, d, *J* 1.8, 8.2 Hz, H-6), 7.74 (1H, d, *J* 7.2 Hz, ArH), 7.94 (1H, d, *J* 1.8 Hz, H-4), 8.00 (1H, d, *J* 8.0 Hz, ArH), 8.66 (1H, t, *J* 5.4 Hz, HNCH$_2$CH$_3$), 11.27 (1H, s, NHCO), 14.28 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 15.31 (HNCH$_2$CH$_3$), 23.91 (CH(CH$_3$)$_2$), 34.70 (HNCH$_2$CH$_3$), 45.88 (CH(CH$_3$)$_2$), 111.02 (CH, Ar), 115.11 (CH, Ar), 117.59 (CH, Ar), 119.38 (C, Ar), 122.46 (C, Ar). 122.78 (CH, Ar), 127.92 (CH, Ar), 128.48 (C, Ar), 128.84 (CH, Ar), 133.04 (CH, Ar), 135.66 (C, Ar), 143.53 (C=N), 162.75 (C=O), 167.62 (C=O). ν_{max} (solid)/(cm^{-1}) 1683 (st), 1625 (md), 1491 (st), 1309 (md), 1139 (st). MS *m/z* (**API-ES**): found 430 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 430.1538 (M+H)$^+$, calculated for $C_{20}H_{24}N_5O_4S$ 430.1549.

N-Furan-2-ylmethyl-3-[N'-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzamide **(193j)**. This was obtained as a yellow solid (0.072 g, 0.149 mmol, 77%) from **192b** (0.114 g, 0.194 mmol) and furfurylamine (0.028 g, 0.291 mmol) in a similar manner as described for preparation of **193a**, mp 230 °C (dec). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 0.93 (6H, d, *J* 6.8 Hz, CH(CH$_3$)$_2$), 3.16-3.23 (1H, s, CH(CH$_3$)$_2$), 4.50 (1H, d, *J* 5.5 Hz, CH$_2$), 6.29 (1H, d, *J* 2.8 Hz, ArH), 6.38-6.40 (1H, m, ArH), 7.07 (1H, d, *J* 8.2 Hz, H-7), 7.46-7.50 (2H, m, ArH & NHSO$_2$), 7.55-7.57 (2H, m, ArH), 7.63 (1H, d, *J* 8.4 Hz, ArH), 7.68 (1H, dd, *J* 1.5, 8.2 Hz, H-6), 7.94 (1H, d, *J* 1.5 Hz, H-4), 7.96 (1H, s, ArH), 8.45 (1H, d, *J* 4.3 Hz, ArH), 8.55 (1H, s, ArH), 9.05 (1H, t, *J* 5.5 Hz, CONH), 11.44 (1H, s, NHCO), 12.80 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 23.89 (CH(CH$_3$)$_2$), 36.80 (CH$_2$), 45.89 (CH(CH$_3$)$_2$), 107.64 (CH, Ar), 111.18 (CH, Ar), 111.23 (CH, Ar), 113.97 (CH, Ar), 117.52 (CH, Ar), 118.00 (CH, Ar), 122.144 (C, Ar), 122.87 (C, Ar), 127.72 (C, Ar), 127.89 (CH, Ar), 130.24 (CH, Ar), 135.91 (C, Ar), 136.18 (C, Ar), 142.71 (CH, Ar),

143.05 (CH, Ar), 143.15 (C=N), 153.01 (CH, Ar), 163.91 (C=O), 166.39 (C=O). ν_{max} (solid)/(cm^{-1}) 3400 (md), 3273 (md), 1694 (st), 1555 (st), 1304 (st), 1240 (md), 1136 (st), 1068 (st). MS *m/z* (**API-ES**): found 482 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 482.1489 $(M+H)^+$, calculated for $C_{23}H_{24}N_5O_5S$ 482.1498.

3-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzamide **(193k)**. This was obtained as a yellow solid (0.045 g, 0.112 mmol, 54%) from **192b** (0.123 g, 0.209 mmol) and ammonia (2M in ethanol) (0.157 ml, 0.314 mmol) in a similar manner as described for preparation of **193a**, mp 250 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.0 Hz, CH(C<u>H</u>$_3$)$_2$), 3.19-3.23 (1H, m, C<u>H</u>(CH$_3$)$_2$), 7.03 (1H, d, *J* 8.3 Hz, H-7), 7.44 (1H, t, *J* 7.8 Hz, ArH), 7.44 (1H, bs, CONH), 7.49 (1H, d, *J* 7.8 Hz, ArH), 7.56 (1H, d, *J* 7.8 Hz, ArH), 7.61 (1H, d, *J* 7.8 Hz, ArH), 7.68 (1H, dd, *J* 1.4, 8.3 Hz, H-6), 7.95 (1H, d, *J* 1.4 Hz, H-4), 7.96 (1H, s, ArH), 8.05 (1H, bs, CONH), 11.43 (1H, s, N<u>H</u>CO), 12.79 (1H, s, NN<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.89 (CH(<u>C</u>H$_3$)$_2$), 45.88 (<u>C</u>H(CH$_3$)$_2$), 111.22 (CH, Ar), 114.10 (CH, Ar), 117.49 (CH, Ar), 118.02 (CH, Ar), 122.18 (C, Ar), 123.04 (CH, Ar), 127.63 (C, Ar), 127.88 (CH, Ar), 130.15 (CH, Ar), 135.91 (C, Ar), 136.39 (C, Ar), 143.01 (C, Ar), 143.12 (C=N), 163.90 (C=O), 168.21 (C=O). ν_{max} (solid)/(cm^{-1}) 1698 (st), 1642 (md), 1564 (st), 1234 (md), 1161 (st), 1122 (st), 1074 (md). MS *m/z* (**API-ES**): found 402 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 402.1234 $(M+H)^+$, calculated for $C_{18}H_{20}N_5O_4S$ 402.1236.

N-(2-Dimethylamino-ethyl)-3-[N'-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzamide **(193l)**. This was obtained as a yellow solid (0.043 g, 0.091 mmol, 39%) from **192b** (0.137 g, 0.350 mmol) and *N,N*-dimethylethylenediamine (0.030 g, 0.350 mmol) in a similar manner as described for preparation of **193a**, mp 260-262 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.4 Hz, CH(C<u>H</u>$_3$)$_2$), 2.19 (6H, s, N(C<u>H</u>$_3$)$_2$), 2.42 (2H, t, *J* 6.6 Hz, CONHCH$_2$C<u>H</u>$_2$), 3.17-3.24 (1H, m, C<u>H</u>(CH$_3$)$_2$), 7.07 (1H, d, *J* 8.4 Hz, H-7), 7.43-7.53 (3H, m, 2 x ArH & NHSO$_2$), 7.62 (1H, d, *J* 8.0 Hz, ArH), 7.68 (1H, dd, *J* 1.6, 8.4 Hz, H-6), 7.92 (1H, s, ArH), 7.95 (1H, d, *J* 1.8 Hz, H-4), 8.47 (1H, t, *J* 5.4 Hz, CONH), 11.45 (1H, s, N<u>H</u>CO), 12.80 (1H, s, NN<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.90 (CH(<u>C</u>H$_3$)$_2$), 38.06 (CH$_2$), 45.85 (N(<u>C</u>H$_3$)$_2$), 45.98 (<u>C</u>H(CH$_3$)$_2$), 58.77 (CH$_2$), 111.25 (CH, Ar), 113.83 (CH, Ar), 117.49 (CH, Ar), 117.79 (CH, Ar), 122.13 (C, Ar), 122.72 (CH, Ar), 127.69 (C, Ar), 127.88 (CH, Ar), 130.19 (CH, Ar), 135.92 (C, Ar), 136.62 (C, Ar), 143.01 (C, Ar), 143.14 (C=N), 163.92 (C=O), 166.40 (C=O). ν_{max} (solid)/(cm^{-1}) 3403 (md), 3283 (md), 1696 (st), 1636 (md), 1554 (st), 1492 (md), 1309 (st), 1157 (st), 1136 (st), 1068 (st). MS *m/z* (**API-ES**): found 473 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 473.1976 $(M+H)^+$, calculated for $C_{22}H_{29}N_6O_4S$ 473.1971.

3-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]-N-(2-methoxyethyl)benzamide **(193m)**. This was obtained as a yellow solid (0.050 g, 0.108 mmol, 46%) from **192b** (0.140 g, 0.238 mmol) and 2-methoxyethylamine (0.026 g, 0.357 mmol) in a similar manner as described for preparation of **193a**, mp 275-277 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 5.6 Hz, CH(C$\underline{H}_3$)$_2$), 3.26 (3H, s, OCH_2), 3.39-3.45 (4H, m, $COCH_2CH_2$), 7.08 (1H, d, *J* 8.2 Hz, H-7), 7.44-7.55 (3H, m, 2 x ArH & $NHSO_2$), 7.62 (1H, d, *J* 7.6 Hz, ArH), 7.69 (1H, dd, *J* 8.2 Hz, H-6), 7.95 (2H, s, ArH & H-4), 8.61 (1H, bs, CONH), 11.44 (1H, s, N$\underline{H}$CO), 12.81 (1H, s, NN$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.89 (CH(C$\underline{H}_3$)$_2$), 45.89 (C$\underline{H}$(CH$_3$)$_2$), 58.61 (OCH_3), 71.11 (CH_2), 111.24 (CH, Ar), 113.88 (CH, Ar), 117.50 (CH, Ar), 117.83 (CH, Ar), 122.13 (C, Ar), 122.78 (CH, Ar), 127.68 (C, Ar), 127.88 (CH, Ar), 130.20 (CH, Ar), 135.91 (C, Ar), 136.48 (C, Ar), 143.01 (C, Ar), 143.12 (C=N), 163.91 (C=O), 166.55 (C=O). ν_{max} (solid)/(cm^{-1}) 3404 (md), 3277 (md), 1694 (st), 1638 (md), 1555 (st), 1490 (md), 1309 (st), 1242 (md), 1156 (st), 1135 (st). MS *m/z* (**API-ES**): found 460 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 460.1649 $(M+H)^+$, calculated for $C_{21}H_{26}N_5O_5S$ 460.1655.

N-Benzyl-3-[N'-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzamide **(193n)**. This was obtained as a yellow solid (0.079 g, 0.160 mmol, 70%) from **192b** (0.136 g, 0.231 mmol) and benzylamine (0.037 g, 0.347 mmol) in a similar manner as described for preparation of **193a**, mp 286-288 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 7.2 Hz, CH(C$\underline{H}_3$)$_2$), 3.16-3.25 (1H, s, C$\underline{H}$(CH$_3$)$_2$), 4.48 (1H, d, *J* 5.7 Hz, CH_2), 7.07 (1H, d, *J* 8.2 Hz, H-7), 7.22-7.25 (1H, m, ArH), 7.29-7.33 (4H, m, ArH), 7.45-7.50 (2H, m, 2 x ArH & $NHSO_2$), 7.59 (1H, d, *J* 8.0 Hz, ArH), 7.64 (1H, d, *J* 8.0 Hz, ArH), 7.69 (1H, dd, *J* 1.7, 8.2 Hz, H-6), 7.95 (1H, d, *J* 1.7 Hz, H-4), 7.98 (1H, s, ArH), 9.13 (1H, t, *J* 5.7 Hz, CONH), 11.44 (1H, s, N$\underline{H}$CO), 12.81 (1H, s, NN$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.90 (CH(C$\underline{H}_3$)$_2$), 43.41 (CH_2), 45.89 (C$\underline{H}$(CH$_3$)$_2$), 111.23 (CH, Ar), 113.96 (CH, Ar), 117.52 (CH, Ar), 117.93 (CH, Ar), 122.15 (C, Ar), 122.82 (CH, Ar), 127.44 (CH, Ar), 127.72 (C, Ar), 127.89 (CH, Ar), 127.95 (2 x CH, Ar), 128.98 (2 x CH, Ar), 130.27 (CH, Ar), 135.94 (C, Ar), 136.42 (C, Ar), 140.27 (C, Ar), 143.07 (C, Ar), 143.15 (C=N), 163.92 (C=O), 166.52 (C=O). ν_{max} (solid)/(cm^{-1}) 3402 (md), 3274 (md), 1697 (st), 1554 (st), 1303 (st), 1155 (st), 1135 (st), 1068 (st). MS *m/z* (**API-ES**): found 492 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 492.1691 $(M+H)^+$, calculated for $C_{25}H_{26}N_5O_4S$ 492.1706.

3-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]-N-pyridin-3-ylmethylbenzamide **(193o).** This was obtained as a yellow solid (0.060 g, 0.121 mmol, 70%) from **192b** (0.102 g, 0.173 mmol) and 3-(aminomethyl)pyridine (0.028 g, 0.260 mmol) in a similar manner as described for preparation of **193a**, mp 260 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.8 Hz, CH(C$\underline{H}_3$)$_2$), 3.16-3.25 (1H, s, C$\underline{H}$(CH$_3$)$_2$), 4.50 (1H, d, *J*

5.8 Hz, CH_2), 7.07 (1H, d, *J* 8.3 Hz, H-7), 7.35 (1H, dd, *J* 4.3, 7.7 Hz, H-6), 7.43-7.50 (2H, m, ArH & $NHSO_2$), 7.57 (1H, d, *J* 8.0 Hz, ArH), 7.64 (1H, d, *J* 8.0 Hz, ArH), 7.68 (1H, dd, *J* 1.5, 8.3 Hz, H-6), 7.73 (1H, d, *J* 7.7 Hz, ArH), 7.94 (1H, d, *J* 1.5 Hz, H-4), 7.96 (1H, s, ArH), 8.45 (1H, d, *J* 4.3 Hz, ArH), 8.55 (1H, s, ArH), 9.18 (1H, t, *J* 5.8 Hz, CONH), 11.43 (1H, s, NHCO), 12.81 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.90 ($CH(CH_3)_2$), 41.19 (CH_2),, 45.88 ($CH(CH_3)_2$), 111.23 (CH, Ar), 113.93 (CH, Ar), 117.50 (CH, Ar), 118.07 (CH, Ar), 122.13 (C, Ar), 122.80 (CH, Ar), 124.20 (CH, Ar), 127.74 (C, Ar), 127.91 (CH, Ar), 130.31 (CH, Ar), 135.72 (CH, Ar), 135.87 (C, Ar), 135.91 (C, Ar), 136.19 (C, Ar), 143.10 (C, Ar), 143.15 (C=N), 148.80 (CH, Ar), 149.56 (CH, Ar), 163.89 (C=O), 166.68 (C=O). ν_{max} (solid)/(cm^{-1}) 3178 (md), 1695 (st), 1638 (md), 1552 (st), 1487 (md), 1301 (st), 1242 (md), 1156 (st), 1118 (st), 1070 (st). MS *m/z* (**API-ES**): found 493 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 493.1655 $(M+H)^+$, calculated for $C_{24}H_{25}N_6O_4S$ 493.1658.

3-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]-N-(2-morpholin-4-yl-ethyl)benzamide **(193p)**. This was obtained as a yellow solid (0.060 g, 0.116 mmol, 57%) from **192b** (0.130 g, 0.204 mmol) and 2-morpholin-4-yl-ethylamine (0.039 g, 0.306 mmol) in a similar manner as described for preparation of **193a**, mp 270 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.4 Hz, $CH(CH_3)_2$), 2.41 (4H, bs, 2 x CH_2), 3.16-3.23 (1H, s, $CH(CH_3)_2$), 3.56 (4H, t, *J* 4.4 Hz, 2 x CH_2), 4.50 (1H, d, *J* 5.5 Hz, CH_2), 7.07 (1H, d, *J* 8.4 Hz, H-7), 7.43-7.52 (3H, m, 2 x ArH & $NHSO_2$), 7.61 (1H, d, *J* 8.0 Hz, ArH), 7.68 (1H, dd, *J* 1.6, 8.4 Hz, H-6), 7.91 (1H, s, ArH), 7.94 (1H, d, *J* 1.6 Hz, H-4), 9.05 (1H, t, *J* 5.6 Hz, CONH), 11.44 (1H, s, NHCO), 12.81 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.90 ($CH(CH_3)_2$), 37.29 (CH_2), 45.88 ($CH(CH_3)_2$), 53.98 (2 x CH_2), 58.00 (CH_2), 66.87 (2 x CH_2), 111.25 (CH, Ar), 113.80 (CH, Ar), 117.48 (CH, Ar), 117.82 (CH, Ar), 122.13 (C, Ar), 122.70 (CH, Ar), 127.69 (C, Ar), 127.88 (CH, Ar), 130.21 (CH, Ar), 135.91 (C, Ar), 136.65 (C, Ar), 143.02 (C, Ar), 143.14 (C=N), 163.91 (C=O), 166.46 (C=O). ν_{max} (solid)/(cm^{-1}) 3411 (md), 3399 (md), 1695 (st), 1636 (md), 1555 (st), 1492 (md), 1301 (st), 1164 (st), 1115 (st), 1068 (st). MS *m/z* (**API-ES**): found 515 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 515.2071 $(M+H)^+$, calculated for $C_{24}H_{31}N_6O_5S$ 515.2077.

3-[N'-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]-N-methylbenzamide **(193q)**. This was obtained as a yellow solid (0.069 g, 0.172 mmol, 79%) from **192b** (0.127 g, 0.216 mmol) and methylamine (40% solution in water) (0.028 ml, 0.324 mmol) in a similar manner as described for preparation of **193a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.0 Hz, $CH(CH_3)_2$), 2.79 (3H, d, *J* 4.4 Hz, NCH_3), 3.16-3.24 (1H, s, $CH(CH_3)_2$), 7.07 (1H, d, *J* 8.2 Hz, H-7), 7.43-752 (3H, m, 2 x ArH & $NHSO_2$), 7.60 (1H, d, *J* 7.6 Hz, ArH), 7.68 (1H, dd, *J* 1.4, 8.2 Hz, H-6), 7.92 (1H, s, ArH), 7.95 (1H, d, *J* 1.4 Hz, H-4), 8.52 (1H, bq, *J* 4.4 Hz, CONH), 11.44 (1H, s, NHCO), 12.81 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.90 ($CH(CH_3)_2$), 26.98 (CH_3), 45.88 ($CH(CH_3)_2$),

111.23 (CH, Ar), 113.68 (CH, Ar), 117.50 (CH, Ar), 117.80 (CH, Ar), 122.15 (C, Ar), 122.62 (CH, Ar), 127.68 (C, Ar), 127.88 (CH, Ar), 130.21 (CH, Ar), 135.93 (C, Ar), 136.65 (C, Ar), 143.06 (C, Ar), 143.13 (C=N), 163.91 (C=O), 166.94 (C=O). ν_{max} (solid)/(cm^{-1}) 1667 (st), 1548 (st), 1467 (st), 1310 (md), 1143 (st). MS *m/z* (**API-ES**): found 416 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 416.1389 $(M+H)^+$, calculated for $C_{19}H_{22}N_5O_4S$ 416.1392.

N-Ethyl-3-[N'-(5-isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]benzamide (**193r**). This was obtained as a yellow solid (0.061, 0.154 mmol, 72%) from **192b** (0.126 g, 0.214 mmol) and ethylamine (60% solution in water) (0.026 ml, 0.312 mmol) in a similar manner as described for preparation of **193a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.0 Hz, $CH(C\underline{H}_3)_2$), 1.12 (3H, t, *J* 7.0 Hz, $NCH_2C\underline{H}_3$), 7.07 (1H, d, *J* 8.2 Hz, H-7), 7.43-753 (3H, m, 2 x ArH & $NHSO_2$), 7.61 (1H, d, *J* 6.8 Hz, ArH), 7.68 (1H, dd, *J* 8.2 Hz, H-6), 7.92 (1H, s, ArH), 7.95 (1H, s, H-4), 8.54 (1H, bs, CONH), 11.44 (1H, s, N<u>H</u>CO), 12.81 (1H, s, NN<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.44 ($CH_2\underline{C}H_3$), 23.90 ($CH(\underline{C}H_3)_2$), 34.81 ($\underline{C}H_2CH_3$), 45.89 ($\underline{C}H(CH_3)_2$), 111.22 (CH, Ar), 113.82 (CH, Ar), 117.49 (CH, Ar), 117.69 (CH, Ar), 122.15 (C, Ar), 122.71 (CH, Ar), 127.66 (C, Ar), 127.87 (CH, Ar), 130.16 (CH, Ar), 135.93 (C, Ar), 136.79 (C, Ar), 142.99 (C, Ar), 143.12 (C=N), 163.92 (C=O), 166.21 (C=O). ν_{max} (solid)/(cm^{-1}) 3396 (md), 3269 (md), 1692 (st), 1613 (md), 1560 (st), 1538 (st), 1489 (md), 1299 (st), 1150 (st), 1070 (st). MS *m/z* (**API-ES**): found 430 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 430.1546 $(M+H)^+$, calculated for $C_{20}H_{24}N_5O_4S$ 430.1549.

2-Oxo-3-(phenylhydrazono)-2,3-dihydro-1H-indole-5-sulfonic acid **(193a)**

A mixture of 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.092 g, 0.3216 mmol) phenylhydrazine (0.052 g, 0.048 mmol, 1.5 eq) and HCl (aq 4M, 0.8 ml) in ethanol (3 mL) was heated in the CEM microwave at 120 °C for 15 min. The mixture was cooled to room temperature, the yellow precipitate was collected by filtration and dried, to give the pure compound (0.080 g, 0.280 mmol, 87%), mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.83 (1H, d, *J* 8.0 Hz, H-7), 7.01-7.04 (1H, m, H-4'), 7.35 (2H, t, *J* 8.4 Hz, H-4' & H-6'), 7.43 (2H, dd, *J* 1.2, 8.4 Hz, H-3' & H-5'), 7.48 (1H, dd, *J* 1.6, 8.0 Hz, H-6), 7.74 (1H, d, *J* 1.6 Hz, H-4), 11.07 (1H, s, NHCO), 12.67 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.12 (CH, Ar), 114.86 (2 x CH, Ar), 116.80 (CH, Ar), 120.98 (C, Ar), 123.66 (CH, Ar), 126.72 (CH, Ar), 128.26 (C, Ar), 130.21 (2 x CH, Ar), 140.41 (C, Ar), 143.19 (C, Ar), 143.25 (C=N), 164.10 (C=O). ν_{max} (solid)/(cm^{-1}) 3536 (md), 3401 (md), 3174 (md), 1697 (md), 1549 (st), 1492 (md), 1184 (st), 1099 (st), 1036 (st). MS *m/z* (**API-ES**): found 316 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**) found 316.0399 $(M-H)^-$, calculated for $C_{14}H_{10}N_3O_4S$ 316.0392.

2-Oxo-3-[(2-methylphenylhydrazono)]-2,3-dihydro-1H-indole-5-sulfonic acid **(194b).** This was obtained as a yellow solid (0.072 g, 0.240 mmol75%) from 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.092 g, 0.321 mmol) and 2-methylphenylhydrazine (0.076 g, 0.482 mmol) in a similar manner as described for preparation of **194a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (3H, s, CH_3), 6.87 (1H, d, *J* 8.1 Hz, H-7), 6.95 (1H, t, *J* 7.5 Hz, ArH), 7.21 (1H, d, *J* 7.5 Hz, ArH), 7.25 (1H, t, *J* 7.5 Hz, ArH), 7.50 (1H, dd, *J* 1.4, 8.1 Hz, H-6), 7.70 (1H, d, *J* 7.5 Hz, ArH), 7.77 (1H, s, H-4), 11.07 (1H, s, NHCO), 12.67 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.05 ($ArCH_3$), 110.29 (CH, Ar), 113.05 (CH, Ar), 116.85 (CH, Ar), 120.76 (C, Ar), 123.22 (CH, Ar), 123.33 (C, Ar), 126.79 (CH, Ar), 128.10 (CH, Ar), 129.11 (C, Ar), 131.45 (CH, Ar), 140.38 (C, Ar), 140.95 (C, Ar), 143.21 (C=N), 164.53 (C=O). ν_{max} (solid)/(cm^{-1}) 3402 (md), 1671 (md), 1552 (st), 1185 (st), 1094 (st), 1030 (st). MS *m/z* (**API-ES**): found 330 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 330.0560 $(M-H)^-$, calculated for $C_{15}H_{12}N_3O_4S$ 330.0549.

2-Oxo-3-[(2,6-dichlorophenylhydrazono)]-2,3-dihydro-1H-indole-5-sulfonic acid **(194c).** This was obtained as a yellow solid (0.078 g, 0.220 mmol, 78%) from 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.081 g, 0.283 mmol) and 2,6-dichlorophenylhydrazine (0.090 g, 0.424 mmol) in a similar manner as described for preparation of **194a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.86 (1H, d, *J* 8.4 Hz, H-7), 7.17 (1H, t, *J* 7.8 Hz, H-4'), 7.52 (1H, dd, *J* 1.6, 8.4 Hz, H-6), 7.55 (2H, d, *J* 7.8 Hz, H-3' & H-5'), 7.64 (1H, s, H-4), 11.21 (1H, s, NHCO), 12.70 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.41 (CH, Ar), 117.62 (CH, Ar), 120.45 (C, Ar), 125.97 (C, Ar), 126.45 (CH, Ar), 127.61 (CH, Ar), 130.57 (2 x CH, Ar), 130.78 (C, Ar), 136.44 (C, Ar), 140.97 (C, Ar), 143.53 (C=N), 163.99 (C=O). ν_{max} (solid)/(cm^{-1}) 3422 (md), 1684 (md), 1618 (md), 1572 (md), 1559 (md), 1161 (st), 1098 (st), 1031 (st). MS *m/z* (**API-ES**): found 383.9 (M ^{35}Cl-H)$^-$ (100%), 385.9 (M ^{37}Cl-H)$^-$ (70%). HRMS *m/z* (**API-ES**): found 383.9619 $(M-H)^-$, calculated for $C_{14}H_9Cl_2N_3O_4S$ 383.9613.

2-Oxo-3-[(2-ethylphenylhydrazono)]-2,3-dihydro-1H-indole-5-sulfonic acid **(194d).** This was obtained as a yellow solid (0.030 g, 0.095 mmol, 30%) from 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.083 g, 0.482 mmol) and 2-ethylphenylhydrazine (0.092 g, 0.321 mmol) in a similar manner as described for preparation of **194a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (3H, t, *J* 7.4 Hz, $CH_2\underline{CH_3}$), 2.63 (2H, q, *J* 7.4 Hz, $\underline{CH_2}CH_3$), 6.86 (1H, d, *J* 8.1 Hz, H-7), 6.98-7.01 (1H, m, ArH), 7.21 (1H, d, *J* 7.8 Hz, ArH), 7.26 (1H, t, *J* 7.8 Hz, ArH), 7.49 (1H, dd, *J* 1.8, 8.1 Hz, H-6), 7.72 (1H, d, *J* 7.8 Hz, ArH), 7.76 (1H, d, *J* 1.8 Hz, H-4), 11.13 (1H, s, NHCO), 13.03 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.25 ($CH_2\underline{CH_3}$), 23.90 ($\underline{CH_2}CH_3$), 110.24 (CH, Ar), 113.46 (CH, Ar), 116.79 (CH, Ar), 120.74 (C, Ar), 123.61 (CH, Ar), 126.70 (CH, Ar), 128.14 (CH, Ar), 129.08 (C, Ar), 129.15 (C, Ar), 129.71 (CH, Ar), 140.24 (C, Ar), 140.30 (C, Ar), 143.52 (C=N), 164.54 (C=O). ν_{max} (solid)/(cm^{-1}) 3188 (md), 1672 (md), 1552 (st), 1186 (st), 1099 (st), 1036 (st).

MS *m/z* (**API-ES**): found 344 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found: 344.0717 $(M-H)^-$, calculated for $C_{16}H_{14}N_3O_4S$: 344.0705.

2-Oxo-3-[(2-fluorophenylhydrazono)]-2,3-dihydro-1H-indole-5-sulfonic acid **(194e)**. This was obtained as a yellow solid (0.088 g, 0.290 mmol, 93%) from 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.090 g, 0.314 mmol) and 2-fluorophenylhydrazine (0.076 g, 0.471 mmol) in a similar manner as described for preparation of **194a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.86 (1H, d, *J* 8.1 Hz, H-7), 7.00-7.06 (1H, m, ArH), 7.25 (1H, t, *J* 7.6 Hz, ArH), 7.27-7.32 (1H, m, ArH), 7.52 (1H, dd, *J* 1.4, 8.1 Hz, H-6), 7.76-7.81 (2H, m, ArH), 11.21 (1H, s, NHCO), 12.85 (1H, d, *J* 1.6 Hz, NNH). ^{19}F NMR (400 MHz, DMSO-d_6) δ -135.59 (ArF). ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.40 (CH, Ar), 115.10 (CH, Ar), 116.27 (d) (CH-3', *J* 16.8 Hz), 117.27 (CH, Ar), 120 43 (C, Ar), 123.66 (d), (CH-6', *J* 7.4 Hz), 126.31 (d), (CH-4', *J* 2.9 Hz), 130.72 (C, Ar), 130.35 (d) (C-1', *J* 9.5 Hz), 140.83 (C, Ar), 143.50 , (C=N), 150.73 (d) (CF, *J* 239.6 Hz), 164.51 , (C=O). ν_{max} (solid)/(cm^{-1}) 3493 (md), 3430 (md), 1687 (md), 1618 (md), 1556 (md), 1260 (md), 1176 (st), 1094 (st), 1028 (st). MS *m/z* (**API-ES**): found 333.9 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 334.0310 $(M-H)^-$, calculated for $C_{14}H_{10}N_3O_4S$ 334.0298.

2-Oxo-3-[(2-trifluoromethylphenylhydrazono)]-2,3-dihydro-1H-indole-5-sulfonic acid **(194f)**. This was obtained as a yellow solid (0.095 g, 0.269 mmol, 87%) from 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.088g g, 0.307 mmol) and 2-trifluoromethylphenylhydrazine (0.081 g, 0.461 mmol) in a similar manner as described for preparation of **194a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.87 (1H, d, *J* 8.3 Hz, H-7), 7.18 (1H, t, *J* 8.2 Hz, ArH), 7.53 (1H, dd, *J* 1.5, 8.3 Hz, H-6), 7.65-7.71 (2H, m, 2 x ArH), 7.79 (1H, d, *J* 1.5 Hz, H-4), 8.02 (1H, t, *J* 8.2 Hz, ArH), 11.21 (1H, s, NHCO), 13.22 (1H, s, NNH). ^{19}F NMR (400 MHz, DMSO-d_6) δ -60.66 (CF_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.59 (CH, Ar), 113.89 (q), (C-2', *J* 30.0 Hz), 115.49 , (CH, Ar), 117.62 , (CH, Ar), 120.29 (C, Ar), 122.98 , (CH, Ar), 124.91 (q), (CF_3, *J* 260.3 Hz), 127.05 (q), (CH-3', *J* 5.9 Hz), 127.91 , (CH, Ar), 131.74 , (C, Ar), 134.96 , (C, Ar), 140.05 (C, Ar), 141.41 , (C, Ar), 143.48 , (C=N), 164.31 , (C=O). ν_{max} (solid)/(cm^{-1}) 3392 (md), 1687 (md), 1590 (md), 1567 (md), 1460 (md), 1323 (md), 1160 (st), 1096 (st), 1030 (st). MS *m/z* (**API-ES**): found 384 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 384.0279 $(M-H)^-$, calculated for $C_{15}H_{19}F_3N_3O_4S$ 384.0266.

2-Oxo-3-(pentafluorophenylhydrazono)-2,3-dihydro-1H-indole-5-sulfonic acid **(194g)**. This was obtained as a yellow solid (0.54 g, 0.144 mmol, 51%) from 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.081 g, 0.0.283 mmol) and pentafluoromethylphenylhydrazine (0.084 g, 0.424 mmol) in a similar manner as described for preparation of **194a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.87 (1H, d, *J* 7.9 Hz, H-7), 7.53 (1H, dd, *J* 1.7, 7.9 Hz, H-

6), 7.64 (1H, d, *J* 1.7 Hz, H-4), 11.28 (1H, s, NHCO), 12.52 (1H, s, NNH). ^{19}F NMR (400 MHz, DMSO-d_6) δ -165.45 (1F, t, *J* -24.4 Hz, F-4'), (-166.80)-(-163.69) (2F, m, F-3' & F-5-'), 155.80 (2F, d, *J* -24.4 Hz, F-2' & F-6'). ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.65 (CH, Ar), 135.30-136.69 (m) (CF), 137.14-137.13 (m) (2 x CF), , 139.41-140.30 (m) (2 x CF), 117.48 (CH, Ar), 119.45 (C, Ar), 128.211 (CH, Ar), 133.08 (C, Ar), 141.40 (C, Ar), 143.74 (C=N), 164.11 (C=O). ν_{max} (solid)/(cm^{-1}) 3464 (md), 1689 (md), 1523 (st), 1180 (st), 1096 (st), 1030 (st). MS *m/z* (**API-ES**): found 405.9 (M-H)$^-$ (100%). HRMS *m/z* (**API-ES**): found 405.9935 (M-H)$^-$, calculated for $C_{14}H_{16}F_5N_3O_4S$ 405.9921.

3-(Naphthalen-1-ylhydrazono)-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid **(194h)**. This was obtained as a red solid (0.085 g, 0.253 mmol, 78%) from 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.092 g, 0.0.321 mmol) and 1-naphthylhydrazine (0.093 g, 0.482 mmol) in a similar manner as described for preparation of **194a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.89 (1H, d, *J* 8.4 Hz, H-7), 7.52-7.60 (3H, m, 3 x ArH), 7.63-7.68 (2H, m, ArH), 7.83-7.90 (3H, m, ArH), 7.98 (1H, d, *J* 8.0, ArH), 11.29 (1H, s, NHCO), 13.73 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 109.23 (CH, Ar), 110.42 (CH, Ar), 117.13 (CH, Ar), 119.67 (CH, Ar), 120.61 (C, Ar), 122.28 (C, Ar), 123.35 (CH, Ar), 127.11 (CH, Ar), 127.17 (CH, Ar), 127.18 (CH, Ar), 127.33 (CH, Ar), 129.47 (CH, Ar), 130.41 (C, Ar), 134.47 (C, Ar), 137.67 (C, Ar), 140.61 (C, Ar), 143.56 (C=N), 164.82 (C=O). ν_{max} (solid)/(cm^{-1}) 3433 (md), 1674 (md), 1616 (md), 1560 (st), 1398 (md), 1185 (st), 1158 (st), 1098 (st), 1029 (st). MS *m/z* (**API-ES**): found 366 (M-H)$^-$ (100%). HRMS *m/z* (**API-ES**): found 366.0555 (M-H)$^-$, calculated for $C_{18}H_{12}N_3O_4S$ 366.0549.

2-Oxo-3-[(2,4-dichlorophenylhydrazono)]-2,3-dihydro-1H-indole-5-sulfonic acid **(194i)**. This was obtained as a yellow solid (0.061 g, 0.172 mmol, 69%) from 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.071 g, 0.248 mmol) and 2,4-dichlorophenylhydrazine (0.079 g, 0.372 mmol) in a similar manner as described for preparation of **194a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.86 (1H, d, *J* 8.3 Hz, H-7), 7.43 (1H, dd, *J* 2.2, 8.8 Hz, H-5'), 7.53 (1H, dd, *J* 1.7, 8.3 Hz, H-6), 7.67 (1H, d, *J* 2.2 Hz, H-3'), 7.79 (1H, d, *J* 1.7 Hz, H-4), 7.84 (1H, d, *J* 8.8 Hz, H-6'), 11.24 (1H, s, NHCO), 13.00 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 105.00 (CH, Ar), 110.48 (CH, Ar), 116.15 (C, Ar), 117.56 (CH, Ar), 119.42 (C, Ar), 120.81 (CH, Ar), 126.76 (C, Ar), 127.84 (CH, Ar), 129.49 (C, Ar), 129.57 (C, Ar), 131.75 (CH, Ar), 138.71 (C, Ar), 141.87 (C=N), 164.33 (C=O). ν_{max} (solid)/(cm^{-1}) 3445 (md), 1678 (md), 1575 (md), 1509 (md), 1182 (st), 1097 (st), 1033 (md). MS *m/z* (**API-ES**): found 383.9 (M ^{35}Cl-H)$^-$ (100%), 385.9 (M ^{37}Cl-H)$^-$ (70%). HRMS *m/z* (**API-ES**): found 383.9619 (M-H)$^-$, calculated for $C_{14}H_9Cl_2N_3O_4S$: 383.9613.

2-Oxo-3-[(2,5-dichlorophenylhydrazono)]-2,3-dihydro-1H-indole-5-sulfonic acid **(194j)**. This was obtained as a yellow solid (0.083 g, 0.235 mmol, 76%) from 5-isatinsulfonic acid

sodium salt dihydrate (**188**) (0.088 g, 0307 mmol) and 2,5-dichlorophenylhydrazine (0.098 g, 0.460 mmol) in a similar manner as described for preparation of **194a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.87 (1H, d, *J* 7.8 Hz, H-7), 7.08 (1H, dd, *J* 2.5, 8.5 Hz, H-4'), 7.52 (1H, d, *J* 8.5 Hz, H-3'), 7.55 (1H, dd, *J* 1.4, 7.8 Hz, H-6), 7.70 (1H, d, *J* 2.5 Hz, H-6'), 7.82 (1H, d, *J* 1.4 Hz, H-4), 11.26 (1H, s, NHCO), 13.00 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.56 (CH, Ar), 114.15 (CH, Ar), 117.38 (C, Ar), 117.86 (CH, Ar), 120.12 (C, Ar), 123.30 (CH, Ar), 128.09 (CH, Ar), 131.73 (CH, Ar), 132.28 (C, Ar), 134.04 (C, Ar), 140.61 (C, Ar), 141.50 (C, Ar), 143.50 (C=N), 164.32 (C=O). ν_{max} (solid)/(cm^{-1}) 3454 (md), 1684 (md), 1568 (md), 1226 (st), 1164 (st), 1094 (st), 1029 (st). MS *m/z* (**API-ES**): found 383.9 (M ^{35}Cl-H)$^-$ (100%), 385.9 (M ^{37}Cl-H)$^-$ (70%). HRMS *m/z* (**API-ES**): found 383.9618 (M-H)$^-$, calculated for $C_{14}H_9Cl_2N_3O_4S$ 383.9613.

2-[N'-(2-Oxo-5-sulfo-1,2-dihydro-indol-3-ylidene)hydrazino]benzoic acid **(194k)**

A mixture of 5-isatinsulfonic acid sodium salt dihydrate (**188**) (0.112 g, 0.391 mmol) 2-carboxylphenylhydrazine (0.052 g, 0.048 mmol) and HCl (aq 4M, 0.7 ml) in ethanol (3 mL) was heated in the CEM microwave at 180 °C for 5 min. The reaction mixture was cooled to room temperature, and the yellow precipitate was collected by filtration and dried., to give pure **194k** (0.116 g, 0.321 mmol, 82%), mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82 (1H, d, *J* 8.3 Hz, H-7), 7.05 (1H, t, *J* 7.8 Hz, ArH), 7.49 (1H, dd, *J* 1.5, 8.3 Hz, H-6), 7.59-7.63 (1H, m, ArH), 7.77 (1H, d, *J* 1.5 Hz, H-4), 7.91 (1H, dd, *J* 1.8, 7.8 Hz, ArH), 7.77 (1H, d, *J* 7.8 Hz, ArH), 11.96 (1H, s, NHCO), 13.73 (1H, s, COOH), 14.15 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.11 (CH, Ar), 114.34 (C, Ar), 114.67 (CH, Ar), 117.37 (CH, Ar), 120.99 (C, Ar), 122.13 (CH, Ar), 127.42 (CH, Ar), 130.67 (C, Ar), 131.97 (CH, Ar), 135.23 (CH, Ar), 141.27 (C, Ar), 143.15 (C, Ar), 145.45 (C=N), 163.07 (C=O), 168.91 (C=O). ν_{max} (solid)/(cm^{-1}) 3185 (md), 1705 (md), 1688 (md), 1513 (md), 1503 (md), 1227 (st), 1183 (st), 1154 (st), 1094 (st), 1035 (st). MS *m/z* (**API-ES**): found 360 (M-H)$^-$ (100%). HRMS *m/z* (**API-ES**): found: 360.0297 (M-H)$^-$, calculated for $C_{15}H_{10}N_3O_6S$ 360.0290.

2-Oxo-2,3-dihydro-1H-indole-5-sulfonyl chloride **(196)**[395]

Oxindole (**195**) (5.5 g, 41.30 mmol) was added portionwise to chlorosulfonic acid (50 ml) maintaining the temperature below 30 °C during the addition. After the addition the reaction mixture was stirred at room temperature for 1.5 h and then at 70 °C for 1 h. After cooling to room temperature, the reaction mixture was poured into ice-water (200 ml) and the pink precipitate was filtered, washed with water (50 ml) and dried, to give pure **196** (8.4 g, 36.36 mmol, 88 %), mp 280-282 °C. ^{1}H NMR (400 MHz, CD_3CN) δ 3.59 (2H, s, CH_2), 7.10 (1H, d, *J* 8.7 Hz, H-7), 7.92 (1H, s, H-4), 7.95 (1H, dd, *J* 2.2, 8.7 Hz, H-6), 8.95 (1H, s, NH).

***2-Oxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide* (197a).** This was obtained from **196** (0.600 g, 2.597 mmol) and isopropylamine (0.184 g, 0.265 mmol) in a similar manner as described for preparation of **190r**. The pure compound was obtained as a pink solid (0.600 g, 2.360 mmol, 91%) without further purification, mp 233-235 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (6H, d, *J* 8.8 Hz, CH(C$\underline{H_3}$)$_2$), 3.15 (1H, sext, *J* 6.6 Hz, C$\underline{H}$(CH$_3$)$_2$), 6.92 (1H, d, *J* 8.3 Hz, H-7), 7.38 (1H, d, *J* 6.6 Hz, $NHSO_2$), 7.58 (1H, s, H-4), 7.61 (1H, dd, *J* 2.2, 8.3 Hz, H-6), 10.74 (1H, s, NH).

***2-Oxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chloro-benzylamide* (197b).** This was obtained from **196** (0.370 g, 1.60 mmol) and 4-chlorobenzylamine (0.271 g, 1.922 mmol) in a similar manner as described for preparation of **190r**. The pure compound was obtained as a pink solid (0.442 g, 1.315 mmol, 82%) without further purification, mp 145-147 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.53 (2H, s, CH_2), 3.92 (2H, s, NCH_2), 6.90 (1H, d, *J* 8.0 Hz, H-7), 7.21 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.30 (2H, d, *J* 8.4 Hz, 2 x CH, Ar), 7.41 (1H, s, $NHSO_2$), 7.51 (1H, s, H-4), 7.61 (1H, d, *J* 8.0 Hz, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.23 (CH_2), 46.07 (NCH_2), 109.55 (CH, Ar), 123.47 (CH, Ar), 127.22 (C, Ar), 127.85 (CH, Ar), 128.76 (2 x CH, Ar), 130.13 (2 x CH, Ar), 132.29 (C, Ar), 133.74 (C, Ar), 137.52 (C, Ar), 148.03 (C, Ar), 177.11 (C=O). ν_{max} (solid)/(cm^{-1}) 3149 (md), 1686 (st), 1617 (md), 1478 (md), 1329 (md), 1149 (st). MS *m/z* (**API-ES**): found 337 $(M^{35}C+H)^+$(100%), 339 $(M^{37}C+H)^+$(35%) HRMS *m/z* (**API-ES**): found 337.0411 $(M+H)^+$, calculated for $C_{15}H_{14}ClN_2O_3S$ 337.0414.

***3-Dimethylaminomethylene-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide* (188a)[394]**

A solution of **197a** (1.50 g g, 5.90 mmol) and *N,N*-dimethylformamidedimethylacetal (1.129 g, 7.67 mmol) in DMF (10 ml) was stirred for 1 h at room temperature. Water (20 ml) was added and the product extracted with ethyl acetate (3 x 10 ml). The organic extracts were collected, dried over Na_2SO_4 and the solvent evaporated under reduced pressure to give a yellow solid (1.098 g, 3.55 mmol, 60%), which was used in the next step without further purification.

***3-Dimethylaminomethylene-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide* (198b).** This was obtained from **197b** (0.208 g, 0.619 mmol) and *N,N*-dimethylformamide dimethylacetal (0.118 g, 0.137 mmol) in a similar manner as described for preparation of **198a**. The crude (0.188 g, 0.480 mmol, 78%) was used in next step without further purification.

2-[(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)amino]benzoic acid methyl ester (199a)

A mixture of **198a** (0.260 g, 0.841 mmol) methyl -2-aminobenzoate (**148a**) (0.139 g, 0.925 mmol) and methansulfonic acid (0.088 g, 0.925 mmol) in ethanol (5 mL) was heated in the microwave at 150 °C for 5 min. The reaction mixture was cooled to 0 °C, and the orange precipitate was collected by filtration and dried, to give pure **199a** (0.207 g, 0.498 mmol, 59 %), mp 283-285 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.8 Hz, CH(C$\underline{H}_3$)$_2$), 3.21-3.26 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 3.92 (1H, s, OCH$_3$), 6.97 (1H, d, *J* 7.9 Hz, H-7), 7.16 (1H, t, *J* 8.0 Hz, ArH), 7.25 (1H, d, *J* 7.6 Hz, HNSO$_2$), 7.48 (1H, dd, *J* 1.7, 7.9 Hz, H-6), 7.64-7.69 (1H, m, CH, Ar), 7.98 (1H, dd, *J* 1.4, 8.0 Hz, ArH), 8.01 (1H, d, *J* 8.0 Hz, ArH), 8.20 (1H, d, *J* 1.7 Hz, H-4), 8.89 (1H, d, *J* 12.2 Hz, C=C$\underline{H}$NH), 10.83 (1H, s, 1H, NHCO), 12.39 (1H, d, *J* 12.2 Hz, C=CHN$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.96 (CH(C$\underline{H}_3$)$_2$), 45.75 (C$\underline{H}$(CH$_3$)$_2$), 52.54 (OCH$_3$), 101.50 (C, Ar), 109.53 (CH, Ar), 116.01 (CH, Ar), 117.00 (C, Ar), 117.15 (CH, Ar), 123.01 (CH, Ar), 123.98 (CH, Ar), 125.17 (C, Ar), 132.56 (CH, Ar), 134.03 (C, Ar), 134.80 (CH, Ar), 138.37 (CH, Ar), 141.04 (C, Ar), 143.45 (C=N), 169.84 (C=O), 170.01 (C=O). ν_{max} (solid)/(cm^{-1}) 3256 (md), 1681 (st), 1617 (md), 1583 (st), 1430 (md), 1362 (md), 1300 (md), 1254 (st), 1193 (st), 1137 (st), 1119 (st), 1071 (st), 1008 (md). MS *m/z* (**API-ES**): found 416 (M+H)$^+$(100%). HRMS *m/z* (**API-ES**): found 416.1276 (M+H)$^+$, calculated for $C_{20}H_{22}N_3O_5S$ 416.1280.

3-[(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)amino]benzoic acid ethyl ester **(199b)**. This was obtained as a yellow solid (0.130 g, 0.303 mmol, 47%) from **198a** (0.199 g, 0.644 mmol) and ethyl-3-aminobenzoate (0.106 g, 0.644 mmol) in a similar manner as described for preparation of **199a**, mp 242-244 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (6H, d, *J* 6.4 Hz, CH(C$\underline{H}_3$)$_2$), 1.33 (3H, t, *J* 7.0 Hz, CH$_2$C$\underline{H}_3$), 3.13-3.27 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 4.33 (2H, q, *J* 7.0 Hz, C$\underline{H}_2$CH$_3$), 6.98 (1H, d, *J* 7.9 Hz, H-7), 7.25 (1H, d, *J* 7.6 Hz, HNSO$_2$), 7.48 (1H, dd, *J* 1.7, 7.9 Hz, H-6), 7.51 (1H, t, *J* 8.0 Hz, ArH), 7.65 (1H, d, *J* 8.0 Hz, ArH), 7.74 (1H, dd, *J* 1.8, 8.0 Hz, ArH), 7.97 (1H, s, ArH), 8.14 (1H, d, *J* 1.7 Hz, H-4), 8.93 (1H, d, *J* 13.2 Hz, C=C$\underline{H}$NH), 10.90 (1H, d, *J* 13.2 Hz, C=CHN$\underline{H}$), 10.92 (1H, s, 1H, s, NHCO). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.87 (CH$_2$$\underline{C}H_3$), 23.96 (CH(C$\underline{H}_3$)$_2$), 45.75 (C$\underline{H}_2CH_3$), 61.67 (C$\underline{H}$(CH$_3$)$_2$), 99.93 (C, Ar), 109.77 (CH, Ar), 116.84 (CH, Ar), 117.44 (CH, Ar), 121.55 (CH, Ar), 123,67 (CH, Ar), 124.63 (CH, Ar), 124.95 (C, Ar), 130.70 (CH, Ar), 132.10 (C, Ar), 134.38 (C, Ar), 140.74 (C, Ar), 140.76 (CH, Ar), 140.91 (C, Ar), 166.10 (C=O), 170.66 (C=O). ν_{max} (solid)/(cm^{-1}) 3262 (md), 3128 (md), 1722 (md), 1671 (st), 1654 (st), 1584 (st), 1311 (md), 1281 (st), 1199 (st). MS *m/z* (**API-ES**): found 430 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 430.1435 (M+H)$^+$, calculated for $C_{21}H_{23}N_3O_5S$ 430.1437.

4-[(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)amino]benzoic acid ethyl ester **(199c)**. This was obtained as a yellow solid (0.057 g, 0.132 mmol, 43%) from **198a** (0.098 g, 0.317 mmol) and ethyl -4-aminobenzoate (0.052 g, 0.317 mmol) in a similar manner as described for preparation of **199a**, mp 273-275 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.4 Hz, CH(C$\underline{H}_3$)$_2$), 1.30 (3H, t, *J* 7.2 Hz, $CH_2C\underline{H}_3$), 3.20-3.26 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 4.28 (2H, q, *J* 7.2 Hz, C$\underline{H}_2$CH$_3$), 6.99 (1H, d, *J* 7.9 Hz, H-7), 7.27 (1H, d, *J* 7.2 Hz, $HNSO_2$), 7.49 (1H, dd, *J* 1.7, 7.9 Hz, H-6), 7.57 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.93 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 8.15 (1H, d, *J* 1.7 Hz, H-4), 8.93 (1H, d, *J* 12.6 Hz, C=C$\underline{H}$NH), 10.90 (1H, d, *J* 12.6 Hz, C=CHN$\underline{H}$), 10.97 (1H, s, 1H, s, NHCO). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.91 ($CH_2\underline{C}H_3$), 23.97 (CH($\underline{C}H_3$)$_2$), 45.77 ($\underline{C}H_2$CH$_3$), 61.19 ($\underline{C}$H(CH$_3$)$_2$), 101.14 (C, Ar), 109.96 (CH, Ar), 116.56 (2 x CH, Ar), 117.08 (CH, Ar), 124.12 (CH, Ar), 124.66 (C, Ar), 124.89 (C, Ar), 131.57 (2 x CH, Ar), 134.57 (C, Ar), 139.71 (CH, Ar), 140.60 (C, Ar), 144.49 (C, Ar), 165.90 (C=O), 170.65 (C=O). ν_{max} (solid)/(cm^{-1}) 3291 (st), 1693 (st), 1670 (st), 1641 (st), 1601 (st), 1274 (st), 1178 (st), 1152 (st), 1109 (st). MS *m/z* (**API-ES**): found 430 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 430.1432 (M+H)$^+$, calculated for $C_{21}H_{24}N_3O_5S$ 430.1437.

3-[(2-Nitrophenylamino)methylene]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide **(199d)**. This was obtained as a orange solid (0.088 g, 0.218 mmol, 34%) from **198a** (0.203 g, 0.656 mmol) and 2-nitroaniline (0.090 g, 0.656 mmol) in a similar manner as described for preparation of **199a**, mp 295 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.8 Hz, CH(C$\underline{H}_3$)$_2$), 7.01 (1H, d, *J* 8.2 Hz, H-7), 7.23 (1H, t, *J* 8.5 Hz, ArH), 7.30 (1H, d, *J* 6.8 Hz, $HNSO_2$), 7.53 (1H, dd, *J* 1.6, 8.2 Hz, H-6), 7.80 (1H, t, *J* 8.5 Hz, ArH), 8.15 (1H, d, *J* 8.5 Hz, ArH), 8.22 (1H, s, H-4), 8.23 (1H, d, *J* 8.5 Hz, ArH), 8.99 (1H, d, *J* 12.0 Hz, C=C$\underline{H}$NH), 10.98 (1H, s, NHCO), 10.90 (1H, d, *J* 12.0 Hz, C=CHN$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.97 CH($\underline{C}H_3$)$_2$), 45.80 ($\underline{C}$H(CH$_3$)$_2$), 104.20 (C, Ar), 110.14 (CH, Ar), 117.78 (CH, Ar), 117.81 (CH, Ar), 123.17 (CH, Ar), 124.30 (C, Ar), 124.98 (CH, Ar), 127.00 (CH, Ar), 134.72 (C, Ar), 135.96 (C, Ar), 136.87 (CH, Ar), 137.07 (C, Ar), 137.76 (CH, Ar), 141.44 (C, Ar), 170.07 (C=O). ν_{max} (solid)/(cm^{-1}) 3454 (md), 3283 (st), 1689 (st), 1671 (st), 1597 (st), 1578 (st), 1512 (st), 1336 (st), 1169 (st). MS *m/z* (**API-ES**): found 403 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found: 430.1070 (M+H)$^+$, calculated for $C_{18}H_{19}N_4O_5S$ 403.1076.

3-[(2-Nitrophenylaminomethylene]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide **(199e)**. This was obtained as an orange solid (0.046 g, 0.106 mmol, 33%) from **198b** (0.097 g, 0.322 mmol) and 2-nitroaniline (0.045 g, 0.322 mmol) in a similar manner as described for preparation of **199a**, mp 250 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.98 (2H, d, *J* 6.4 Hz, CH_2), 7.00 (1H, d, *J* 8.4 Hz, H-7), 7.20-7.32 (6H, m), 7.53 (1H, dd, *J* 1.6, 8.4 Hz, ArH), 7.82 (1H, t, *J* 8.0 Hz, ArH), 7.95 (1H, t, *J* 6.6 Hz, ArH), 8.15 (1H, d,

J 8.4 Hz, ArH), 8.19 (1H, d, *J* 1.6 Hz, H-4), 8.24 (1H, dd, *J* 1.4, 8.6 Hz, CH-6), 8.98 (1H, d, *J* 12.2 Hz, C=C<u>H</u>NH), 11.05 (1H, s, NHCO), 12.51 (1H, d, *J* 12.2 Hz, C=CHN<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.14 (NCH_2), 105.60 (C, Ar), 109.53 (CH, Ar), 117.56 (CH, Ar), 117.67 (CH, Ar), 123.53 (CH, Ar), 124.32 (C, Ar), 127.00 (CH, Ar), 127.06 (C, Ar), 127.71 (CH, Ar), 128.43 (2 x CH, Ar), 130.34 (2 x CH, Ar), 131.98 (C, Ar), 133.53 (C, Ar), 134.74 (CH, Ar), 137.58 (C, Ar), 138.12 (CH, Ar), 144.80 (C, Ar), 170.09 (C=O). ν_{max} (solid)/(cm^{-1}) 3244 (st), 1685 (st), 1599 (st), 1509 (st), 1339 (st), 1319 (st), 1197 (st), 1147 (st). MS *m/z* (**API-ES**): found 485 ($M^{35}C+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 485.0677 $(M+H)^+$, calculated for $C_{22}H_{18}ClN_4O_5S$ 485.0686.

***3-[(phenylamino)-methylene]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide* (199f)** This was obtained as a orange solid (0.040 g, 0.111 mmol, 70%) from **198a** (0.049 g, 0.159 mmol) and aniline (0.015 g, 0.161 mmol) in a similar manner as described for preparation of **199a**, mp 298-300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.0 Hz, CH(C<u>H</u>$_3$)$_2$), 3.19-3.27 (1H, m, <u>CH</u>(CH$_3$)$_2$), 6.98 (1H, d, *J* 8.0 Hz, H-7), 7.09 (1H, t, *J* 7.2 Hz, H-4'), 7.24 (1H, d, *J* 7.6 Hz, $HNSO_2$), 7.36-7.40 (2H, m, H-3' & H-5'), 7.44-7.47 (3H, m, ArH), 8.11 (1H, d, *J* 2.0 Hz, H-4), 8.88 (1H, d, *J* 12.8 Hz, C=C<u>H</u>NH), 10.79 (1H, d, *J* 12.8 Hz, C=CHN<u>H</u>), 10.89 (1H, s, NHCO). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.97 CH(<u>CH$_3$</u>)$_2$), 45.75 (<u>CH</u>(CH$_3$)$_2$), 99.12 (C, Ar), 109.71 (CH, Ar), 116.51 (2 x CH, Ar), 116.98 (CH, Ar), 123.35 (C, Ar), 124.29 (CH, Ar), 125.08 (C, Ar), 130.32 (2 x CH, Ar), 134.31 (C, Ar), 140.03 (CH, Ar), 140.35 (C, Ar), 140.93 (C, Ar), 170.68 (C=O). ν_{max} (solid)/(cm^{-1}) 3474 (md), 1680 (st), 1617 (md), 1597 (md), 1583 (st), 1483 (md), 1305 (st), 1277 (st), 1202 (md), 1134 (st), 1072 (st), 1013 (st). MS *m/z* (**API-ES**): found 358 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 358.1222 $(M+H)^+$, calculated for $C_{18}H_{20}N_3O_3S$ 358.1225.

***3-[(Naphthylamino)-methylene]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide* (199g)** This was obtained as a orange solid (0.032 g, 0.078 mmol, 49%) from **198a** (0.059 g, 0.159 mmol) and 1-naphthylamine (0.023 g, 0.160 mmol) in a similar manner as described for preparation of **199a**, mp >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.94 (6H, d, *J* 6.8 Hz, CH(C<u>H</u>$_3$)$_2$), 3.21-3.27 (1H, m, <u>CH</u>(CH$_3$)$_2$), 7.05 (1H, d, *J* 8.4 Hz, H-7), 7.28 (1H, d, *J* 7.2 Hz, $HNSO_2$), 7.50 (1H, dd, *J* 1.8, 8.4 Hz, H-6), 7.55-7.61 (2H, m, ArH), 7.63-7.73 (1H, m, ArH), 7.86 (1H, d, *J* 7.9 Hz, ArH), 8.00 (1H, d, *J* 7.9 Hz, ArH), 8.03 (1H, d, *J* 7.9 Hz, ArH), 8.17 (1H, d, *J* 1.8 Hz, H-4), 9.18 (1H, d, *J* 12.0 Hz, C=C<u>H</u>NH), 11.06 (1H, s, NHCO), 11.90 (1H, d, *J* 12.0 Hz, C=CHN<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.98 CH(<u>CH$_3$</u>)$_2$), 45.78 (<u>CH</u>(CH$_3$)$_2$), 100.41 (C, Ar), 110.04 (CH, Ar), 111.40 (CH, Ar), 116.78 (CH, Ar), 120.22 (C, Ar), 123.60 (CH, Ar), 124.18 (CH, Ar), 124.36 (CH, Ar), 124.74 (C, Ar), 126.90 (CH, Ar), 127.33 (C, Ar), 127.61 (C, Ar), 129.50 (C, Ar), 134.53 (CH, Ar), 134.59 (C, Ar), 135.58 (CH, Ar), 140.08 (CH, Ar), 141.72, 171.42 (C=O). ν_{max} (solid)/(cm^{-1}) 1684 (st), 1623 (md), 1579 (st), 1483 (md), 1324 (st), 1221 (st), 1145 (st), 1021 (st). MS

m/z (**API-ES**): found 408 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 408.1378 $(M+H)^+$, calculated for $C_{22}H_{22}N_3O_3S$ 408.1382.

***2-[(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)amino]benzoic acid* (200a)**

A suspension of **199a** (0.155 g, 0.3734 mmol) in methanol (1 mL) and NaOH 1M (1 ml) was heated in the microwave at 150 °C for 5 min. The reaction mixture was cooled to 0 °C, and the solvent distilled under reduced pressure and HCl (aq 4M, 5 ml) added. The orange precipitate was collected by filtration, washed with water (10 ml) and dried, to give pure **200a** (0.139 g, 0.346 mmol, 92 %), mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.4 Hz, $CH(C\underline{H}_3)_2$), 3.20-3.26 (1H, m, $C\underline{H}(CH_3)_2$), 6.96 (1H, d, *J* 8.1 Hz, H-7), 7.13 (1H, t, *J* 7.8 Hz, ArH), 7.24 (1H, d, *J* 6.8 Hz, $HNSO_2$), 7.47 (1H, dd, *J* 1.8, 8.1 Hz, H-6), 7.95-8.00 (2H, m, ArH), 8.19 (1H, s, H-4), 8.86 (1H, d, *J* 12.8 Hz, C=C$\underline{H}$NH), 10.76 (1H, s, NHCO), 12.56 (1H, d, *J* 12.8 Hz, C=CHN$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.96 ($CH(\underline{C}H_3)_2$), 45.77 ($\underline{C}H(CH_3)_2$), 101.71 (C, Ar), 109.61 (CH, Ar), 115.96 (CH, Ar), 116.78 (C, Ar), 116.99 (CH, Ar), 122.91 (CH, Ar), 123.99 (CH, Ar), 125.17 (C, Ar), 132.36 (CH, Ar), 134.26 (C, Ar), 134.92 (CH, Ar), 138.32 (CH, Ar), 140.93 (C, Ar), 142.53 (C=N), 168.84 (C=O), 169.65 (C=O). ν_{max} (solid)/(cm^{-1}) 3274 (md), 1680 (st), 1637 (st), 1588 (st), 1459 (st), 1364 (md), 1314 (st), 1278 (st), 1249 (st), 1135 (st), 1115 (st), 1071 (md), 993 (md), 747 (st), 726 (st). MS *m/z* (**API-ES**): found 402 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 402.1116 $(M+H)^+$, calculated for $C_{19}H_{20}N_3O_5S$ 402.1124.

***3-[(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)amino]benzoic acid* (200b)**. This was obtained as an orange solid (0.032 g, 0.798 mmol, 40%) from **199b** (0.087 g, 0.202 mmol) in a similar manner as described for preparation of **200a**, mp 290 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (6H, d, *J* 6.4 Hz, $CH(C\underline{H}_3)_2$), 3.13-3.27 (1H, m, $C\underline{H}(CH_3)_2$), 6.98 (1H, d, *J* 8.4 Hz, H-7), 7.24 (1H, d, *J* 7.2 Hz, $HNSO_2$), 7.46 (1H, dd, *J* 1.6, 8.4 Hz, H-6), 7.49 (1H, t, *J* 7.6 Hz, CH, Ar), 7.63 (1H, d, *J* 8.0 Hz, ArH), 7.69 (1H, d, *J* 8.4 Hz, ArH), 7.97 (1H, s, ArH), 8.15 (1H, s, H-4), 8.94 (1H, d, *J* 11.8 Hz, C=C$\underline{H}$NH), 10.90 (1H, d, *J* 11.8 Hz, C=CHN$\underline{H}$), 10.91 (1H, s, 1H, s, NHCO), 13.13 (1H, bs, COOH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.96 ($CH(\underline{C}H_3)_2$), 45.76 ($\underline{C}H(CH_3)_2$), 109.76 (CH, Ar), 116.82 (CH, Ar), 117.39 (CH, Ar), 121.43 (CH, Ar), 123.60 (CH, Ar), 124.89 (CH, Ar), 130.57 (CH, Ar), 140.84 (CH, Ar). ν_{max} (solid)/(cm^{-1}) 3269 (md), 1678 (st), 1645 (st), 1572 (st), 1459 (st), 1243 (st), 1119 (st), 1057 (md), 984 (md), 779 (st), 734 (st). MS *m/z* (**API-ES**): found 402 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 402.1114 $(M+H)^+$, calculated for $C_{19}H_{20}N_3O_5S$ 402.1124.

***4-[(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)amino]benzoic acid* (200c)**. This was obtained as an orange solid (0.028 g, 0.698 mmol, 33%) from **199c** (0.091

mmol, 0.212 mmol) in a similar manner as described for preparation of **200a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (6H, d, *J* 6.4 Hz, CH(C$\underline{H}_3$)$_2$), 6.99 (1H, d, *J* 8.1 Hz, H-7), 7.27 (1H, d, *J* 7.2 Hz, HNSO$_2$), 7.47 (1H, dd, *J* 1.8, 8.1 Hz, H-6), 7.55 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 7.93 (2H, d, *J* 8.8 Hz, 2 x CH, Ar), 8.16 (1H, s, H-4), 8.94 (1H, d, *J* 12.2 Hz, C=C$\underline{H}$NH), 10.89 (1H, d, *J* 12.2 Hz, C=CHN$\underline{H}$), 10.96 (1H, s, NHCO), 12.78 (1H, bs, COOH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.98 (CH($\underline{C}$H$_3$)$_2$), 45,77 ($\underline{C}$H(CH$_3$)$_2$), 101.95 (C, Ar), 109.55 (CH, Ar), 116.48 (2 x CH, Ar), 117.03 (C, Ar), 123.33 (CH, Ar), 124.72 (CH, Ar), 125.85 (C, Ar), 127.66 (C, Ar), 131.75 (2 x CH, Ar), 139.85 (C, Ar), 140.56 (CH, Ar), 144.21 (C=N), 167.47 (C=O), 170.64 (C=O). ν_{max} (solid)/(cm^{-1}) 3265 (md), 1676 (st), 1642 (st), 1556 (st), 1457 (st), 1314 (st), 1265 (st), 1109 (st), 801 (st), 756 (st). MS *m/z* (**API-ES**): found 402 (M+H)$^+$ (100%). HRMS *m/z* (**API-ES**): found 402.1119 (M+H)$^+$, calculated for $C_{19}H_{20}N_3O_5S$ 402.1124.

3-Bromo-1H-indole-5-carboxylic acid methyl ester (205)[397]

A solution of Br_2 (13.41g, 74.91 mmol) in DMF (2 ml) was added dropwise to a solution of 5-methylindole-2-carboxilate (**204**) (0.225 g, 1.28 mmol) in DMF (5 ml) at room temperature. The reaction mixture was stirred overnight. Water (10 ml) was added and the mixture was extracted with ethyl acetate (2 x 10 ml). The organic extracts were collected, dried over Na_2SO_4, filtered and the solvent removed under reduced pressure to afford an orange solid. The crude material was used in the next step without further purification.

3,3-Dibromo-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester (206)[397]

N-Bromosuccinimide (0.318 g, 1.842 mmol) was added portionwise within 30 min to a solution of **205** (0.234 g, 0,92 mmol) in isopropanol (350 ml) under Ar. The reaction mixture was then stirred for 1 h. The solvent was removed under reduced pressure and the solid residue was triturated with cold acetone (10 ml) to give pure **206** as a yellow solid (0.125 g, 0.359 mmol, 40%), mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.86 (3H, s, OCH$_3$), 7.05 (1H, d, *J* 8.2 Hz, H-7), 7.96 (1H, dd, *J* 1.6, 8.2 Hz, H-6), 8.05 (1H, s, H-4), 11.71 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.92 (OCH$_3$), 111.96 (CH, Ar), 125.37 (C, Ar), 126.83 (CH, Ar), 132.08 (C, Ar), 134.15 (CH, Ar), 143.28 (C, Ar), 165.88 (C=O), 171.27 (CBr$_2$). ν_{max} (solid)/(cm^{-1}) 3125 (md), 1745 (st), 1698 (st), 1622 (st), 1432 (st), 1280 (st), 1251 (st), 1190 (st), 1127 (st), 985 (md), 941 (md), 845 (st), 809 (st), 757 (st). MS *m/z* (**API-ES**): found (M+H)$^+$ (100%).

3,3-Dibromo-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester (206)

N-Bromosuccinimide (13.41g, 74.91 mmol) was added portionwise to a solution of 5-methylindole-2-carboxylate (**204**) (4.5 g, 25.71 mmol) in isopropanol (350 ml) within 45 minutes under Ar at room temperature. After the addition, the solvent was removed under

reduced pressure and the solid residue was triturated with cold acetone (150 ml) to give the pure product as a yellow solid (4.9 g, 14.08 mmol, 55%).

2,3-Dioxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester (203)

A mixture of **206** (0.055 g, 0.1580 mmol) in methanol (3 ml) and water (1 ml) was heated in the CEM microwave at 150 °C for 5 min. After cooling to room temperature, the orange precipitate was collected by filtration and dried under vacuum. Pure **203** was obtained without further purification (0.026 g, 0.1262 mmol, 80%), mp 248-250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (3H, s, OCH_3), 7.00 (1H, d, *J* 8.2 Hz, H-7), 7.90 (1H, d, *J* 1.8 Hz, H-4), 8.13 (1H, dd, *J* 1.8, 8.2 Hz, H-6), 11.40 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.53 (OCH_3), 112.46 (CH, Ar), 118.58 (C, Ar), 125.57 (C, Ar), 125.81 (CH, Ar), 139.78 (CH, Ar), 154.46 (C, Ar), 160.49 (C=O), 166.17 (C=O), 184.34 (C=O). ν_{max} (solid)/(cm^{-1}) 3280 (md), 1723 (st), 1706 (st), 1662(st), 1634 (st), 1432 (st), 1218 (st), 1105 (st), 978 (st). MS *m/z* (**API-ES**): found 206 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 206.0450 $(M+H)^+$, calculated for $C_{10}H_8NO_4$ 206.0453.

3-[(2-Chlorophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester (207a)

A mixture of **206** (0.025 g, 0.0.0718 mmol) and 2-chlorohydrazine (0.013 g, 0.0718 mmol) in methanol (1.6 ml) and water (0.4 ml) was heated in the CEM microwave at 150 °C for 5 min. After cooling to room temperature, the precipitate was collected by filtration and dried in vacuo, to give pure **207a** (0.016 g, 0.047 mmol, 66 %) as an orange solid, mp 278-280 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (3H, s, OCH_3), 7.36-7.09 (1H, m, ArH), 7.04 (1H, d, *J* 8.2 Hz, H-7), 7.38-7.43 (1H, m, ArH), 7.50 (1H, dd, *J* 1.3, 8.2 Hz, ArH), 7.86 (1H, dd, *J* 1.6, 8.2 Hz, H-6), 7.91 (1H, dd, *J* 1.3, 8.2 Hz, ArH), 8.11 (1H, d, *J* 1.6 Hz, H-4), 11.47 (1H, s, NHCO), 12.96 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.65 (OCH_3), 111.33 , (CH, Ar), 115.15 , (CH, Ar), 119.11 , (C, Ar), 120.40 , (CH, Ar), 121.41 , (C, Ar), 124.02 , (C, Ar), 124.38 , (CH, Ar), 129.30 , (CH, Ar), 129.95 , (C, Ar), 130.24 , (CH, Ar), 131.38 , (CH, Ar), 139.14 , (C, Ar), 144.74 , (C=N), 164.23 (C=O), 166.63 (C=O). ν_{max} (solid)/(cm^{-1}) 3622 (md), 1687 (st), 1617 (md), 1549 (st), 1285 (st), 1247 (st), 1186 (st), 744 (st), 678 (st). MS *m/z* (**API-ES**): found 330 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 330.0644 $(M+H)^+$, calculated for $C_{16}H_{13}ClN_3O_3$ 330.0645.

3-(Phenylhydrazono-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester **(207b)**. This was obtained as a yellow solid (0.016 g, 0.054 mmol, 45%) from **196** (0.045 g, 0.129 mmol) and phenylhydrazine (0.013 g, 0.129 mmol) in a similar manner as described for preparation of **207a**, mp 245-247 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.83 (3H, s, OCH_3), 7.00 (1H, d, *J* 8.2 Hz, H-7), 7.06 (1H, t, *J* 7.7 Hz, H-4'), 7.37 (2H, t, *J* 7.7 Hz, H-3' & H-5'), 7.47 (2H, d, *J* 7.7 Hz, H-2' & H-6'), 7.86 (1H, dd, *J* 1.6, 8.2 Hz, H-6), 8.05 (1H, s, H-4),

11.38 (1H, s, NHCO), 12.69 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.65 (OCH_3), 111.10 (CH, Ar), 115.17 (2 x CH, Ar), 119.81 (CH, Ar), 122.09 (C, Ar), 123.83 (CH, Ar), 124.07 (C, Ar), 127.20 (C, Ar), 130.18 (2 x CH, Ar), 130.64 (CH, Ar), 142.98 (C, Ar), 144.12 , (C=N), 163.98 , (C=O), 166.80 , (C=O). ν_{max} (solid)/(cm^{-1}) 3279 (md), 1701 (md), 1684 (md), 1559 (st), 1286 (md), 1250 (st), 1184 (md). MS *m/z* (**API-ES**): found 296 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 296.1035 $(M+H)^+$, calculated for $C_{16}H_{14}N_3O_3$ 296.1035.

***3-[(2-Trifluoromethylphenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester* (207c)**. This was obtained as a yellow solid (0.019 g, 0.052 mmol, 42%) from **206** (0.043 g, 0.123 mmol) and 2-trifluoromethylphenylhydrazine (0.021 g, 0.123 mmol) in a similar manner as described for preparation of **197a**, mp 269-271 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (3H, s, OCH_3), 7.04 (1H, d, *J* 8.1 Hz, H-7), 7.21 (1H, t, *J* 7.8 Hz, ArH), 7.66-7.73 (2H, m, ArH), 7.92 (1H, dd, *J* 1.4, 8.1 Hz, H-6), 8.04 (1H, d, *J* 7.8 Hz, ArH), 8.11 (1H, d, *J* 1.4 Hz, H-4), 11.53 (1H, s, NHCO), 13.20 (1H, s, NNH). ^{19}F NMR (400 MHz, DMSO-d_6) δ -60.53 (CF_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.73 (OCH_3), 111.55 (CH, Ar), 114.14 (q), (C-2', *J* 30.1 Hz), 115.79 (CH, Ar), 120.68 (CH, Ar), 121.36 (C, Ar), 123.39 (CH, Ar), 124.20 (C, Ar), 124.87 (q), (CF_3, *J* 271.1 Hz), 127.10 (q) (CH-3', *J* 5.2 Hz), 130.84 (C, Ar), 131.82 (CH, Ar), 134.97 (C, Ar), 140.41 (C, Ar), 145.12 (C=N), 164.31 (C=O), 166.64 (C=O). ν_{max} (solid)/(cm^{-1}) 1716 (md), 1687 (md), 1569 (st), 1244 (st), 1107 (st), 765 (st). MS *m/z* (**API-ES**): found 364 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 364.0904 $(M+H)^+$, calculated for $C_{17}H_{13}F_3N_3O_3$ 364.0909.

***3-[(2,6-Dichlorophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester* (208d).** This was obtained as a yellow solid (0.024 g, 0.059 mmol, 66%) from **206** (0.033 g, 0.094 mmol) and 2,6-dichlorophenylhydrazine (0.020 g, 0.094 mmol) in a similar manner as described for preparation of **206**, mp 255-257 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (3H, s, OCH_3), 7.03 (1H, d, *J* 8.4 Hz, H-7), 7.20 (1H, t, *J* 8.3 Hz, H-4'), 7.55 (1H, d, *J* 8.3 Hz, H-3' & H-5'), 7.89 (1H, dd, *J* 1.4, 8.4 Hz, H-6), 7.94 (1H, d, *J* 1.4 Hz, H-4), 11.50 (1H, s, NHCO), 12.67 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 52.70 (OCH_3), 111.41 (CH, Ar), 120.08 (CH, Ar), 121.58 (C, Ar), 124.07 (C, Ar), 126.66 (CH, Ar), 127.01 (C, Ar), 129.67 (C, Ar), 130.51 (2 x CH, Ar), 131.50 (CH, Ar), 136.35 (C, Ar), 144.74 (C=N), 163.89 (C=O), 166.62 (C=O). ν_{max} (solid)/(cm^{-1}) 3257 (st), 1705 (st), 1683 (st), 1621 (st), 1573 (st), 1408 (st), 1294 (st), 1254 (st), 1240 (st), 1158 (md), 769 (st). MS *m/z* (**API-ES**): found 364 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 364.0251 $(M+H)^+$, calculated for $C_{16}H_{12}Cl_2N_3O_3$ 364.0256.

2,3-Dioxo-2,3-dihydro-1H-indole-5-carboxylic acid (209)

A mixture of **206** (0.173 g, 0.497 mmol) in HCl (aq 4 M, 5 ml) was heated in the CEM microwave at 150 °C for 5 min. After cooling to room temperature, the orange precipitate was collected by filtration and dried to afford pure **209** without further purification (0.055 g, 0.290 mmol, 58%), mp 295-297 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.97 (1H, d, *J* 8.2 Hz, H-7), 7.89 (1H, d, *J* 1.8 Hz, H-4), 8.12 (1H, dd, *J* 1.8, 8.2 Hz, H-6), 11.35 (1H, s, NH), 13.03 (1H, bs, CO_2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 112.80 (CH, Ar), 118.56 (C, Ar), 125.75 (C, Ar), 125.80 (CH, Ar), 139.83 (CH, Ar), 154.52 (C, Ar), 160.40 (C=O), 166.11 (C=O), 184.18 (C=O). ν_{max} (solid)/(cm^{-1}) 3068 (st), 2993 (st), 1740 (st), 1702 (st), 1675 (st), 1616 (st), 1405 (st), 1250 (st), 1220 (st), 1119 (st), 930 (st), 865 (st), 748 (st). MS *m/z* (**API-ES**): found 190 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 190.0138 $(M-H)^-$, calculated for $C_9H_4NO_4$ 190.0140.

3-[(2-Chlorophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid (208a)

A mixture of **208** (0.052 g, 0.149 mmol) in HCl (aq 4M, 2 ml) was heated in the CEM microwave at 150 °C for 5 min. After cooling to room temperature, 2-chlorohydrazine (0.026 g, 0.149 mmol) was added to the reaction mixture, which was heated in the microwave at 150 °C for 5 min. After cooling to room temperature, the yellow precipitate was collected by filtration, washed with water (5 ml), cold methanol (2 ml) and dried in vacuo, to give pure hydrazone **208a** (0.039 g, 0.121 mmol, 81%), mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.02 (1H, d, *J* 8.4 Hz, H-7), 7.06 (1H, t, *J* 7.8 Hz, ArH), 7.39 (1H, t, *J* 7.8 Hz, ArH), 7.49 (1H, d, *J* 7.8 Hz, ArH), 7.85-7.90 (2H, m, ArH), 8.11 (1H, s, H-4), 11.49 (1H, s, NHCO), 12.83 (1H, bs, CO_2H), 13.03 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 111.26 (CH, Ar), 115.17 (CH, Ar), 119.08 (CH, Ar), 120.75 (C, Ar), 121.33 (C, Ar), 124.40 (C, Ar), 125.26 (CH, Ar), 129.36 (CH, Ar), 130.22 (CH, Ar), 130.29 (C, Ar), 131.64 (CH, Ar), 139.22 (C, Ar), 144.52 (C=N), 164.33 (C=O), 167.75 (C=O). ν_{max} (solid)/(cm^{-1}) 3167 (md), 3024 (st), 1737 (st), 1687 (st), 1616 (st), 1552 (st), 1421 (md), 1293 (st), 1232 (md), 1205 (md), 1189 (md), 1123 (md), 747 (st), 680 (st). MS *m/z* (**API-ES**): found 313.9 (M ^{35}Cl-H)$^-$ (100%), 316 (M ^{37}Cl-H)$^-$ (70%). HRMS *m/z* (**API-ES**): found 314.0344 $(M-H)^-$, calculated for $C_{15}H_9ClN_3O_3$ 314.0332.

***3-[(2-Chlorophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid* (208a)**

A suspension of **207a** (0.019 g, 0.051 mmol) in methanol (4 mL) and NaOH 1M (1 ml) was heated at 80 °C for 8 h. The reaction mixture was cooled to 0 °C, and the solvent distilled under reduced pressure and HCl (aq 4M, 5 ml) added. The orange precipitate was collected by filtration, washed with water (5 ml) and dried, to give pure **208a** (0.010 g, 0.031 mmol, 61 %).

***3-(Phenylhydrazono)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid* (208b).** This was obtained as a yellow solid (0.035 g, 0.121 mmol, 76%) from **206** (0.056 g, 0.160 mmol) and phenylhydrazine in a similar manner as described for preparation of **208a**, mp 294-296 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.99 (1H, d, *J* 8.2 Hz, H-7), 7.05 (1H, t, *J* 7.8 Hz, H-4'), 7.36 (2H, t, *J* 7.8 Hz, H-3' & H-5'), 7.47 (2H, d, *J* 7.8 Hz, H-2' & H-6'), 7.86 (1H, d, *J* 8.2 Hz, H-6), 8.07 (1H, s, H-4), 11.35 (1H, s, NHCO), 12.69 (1H, s, NNH), 12.77 (1H, s, CO_2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.94 (CH, Ar), 115.11 (2 x CH, Ar), 120.11 (CH, Ar), 121.95 (C, Ar), 124.00 (CH, Ar), 125.01 (C, Ar), 127.39 (C, Ar), 130.18 (2 x CH, Ar), 130.81 (CH, Ar), 143.02 (C, Ar), 143.86 (C=N), 164.03 (C=O), 167.87 (C=O). ν_{max} (solid)/(cm^{-1}) 3147 (md), 1682 (st), 1614 (st), 1551 (st), 1421 (md), 1290 (st), 1263 (st), 1253 (st), 1189 (st), 1122 (md), 751 (st), 689 (st). MS *m/z* (**API-ES**): found 282 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found: 282.0875 $(M+H)^+$, calculated for $C_{15}H_{12}N_3O_3$ 282.0779.

***3-[(2-Fluorophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid* (208c).** This was obtained as a yellow solid (0.021 g, 0.070 mmol, 72%) from **206** (0.034 g, 0.097 mmol) and 2-fluorophenylhydrazine (0.015 g, 0.097 mmol) in a similar manner as described for preparation of **208a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.03 (1H, d, *J* 8.1 Hz, H-7), 7.04-7.09 (1H, m, ArH), 7.25 (1H, t, *J* 7.8 Hz, ArH), 7.32 (1H, dd, *J* 8.2, 11.4 Hz, ArH), 7.52 (1H, t, *J* 8.0 Hz, ArH), 7.89 (1H, dd, *J* 1.8, 8.1 Hz, H-6), 8.11 (1H, s, H-4), 11.49 (1H, s, NHCO), 12.86 (2H, bs, NNH, CO_2H). ^{19}F NMR (400 MHz, DMSO-d_6) δ -135.44 (F). ^{13}C NMR (100 MHz, DMSO-d_6) δ 111.23 (CH, Ar), 115.23 (CH, Ar), 116.32 (d) (CH-3', *J* 17.6 Hz), 120.61 (C, Ar), 121.39 (CH, Ar), 124.03 (d), (CH-4', *J* 7.3 Hz), 125.25 (C, Ar), 126.28 (d) (CH-6', *J* 3.7 Hz), 129.88 (C, Ar) 131.15 (d) (C-1', *J* 9.5 Hz), 131.48 (CH, Ar), 144.30 (C=N), 150.81 (d) (CF, *J* 240.3 Hz), 164.51 (C=O), 167.76 (C=O).ν_{max} (solid)/(cm^{-1}) 3180 (md), 1689 (st), 1616 (st), 1557 (st), 1420 (md), 1293 (st), 1263 (st), 1201 (st). MS *m/z* (**API-ES**): found 298 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found: 298.0639 $(M-H)^-$, calculated for $C_{15}H_9FN_3O_3$ 298.0628.

***3-[(2-Ethylphenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid* (208d).** This was obtained as a yellow solid (0.023 g, 0.071 mmol, 65%) from **206** (0.040 g, 0.114 mmol) and 2-ethylphenylhydrazine (0.019 g, 0.114 mmol) in a similar manner as described for preparation of **208a**, mp 300 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (3H, t, *J* 7.6 Hz, $CH_2\underline{CH_3}$), 2.64 (2H, q, *J* 7.6 Hz, $\underline{CH_2}CH_3$), 7.01-7.04 (1H, m, ArH), 7.03 (1H, d, *J* 8.3 Hz, H-7), 7.23 (1H, d, *J* 7.6 Hz, ArH), 7.28 (1H, t, *J* 7.6 Hz, ArH), 7.75 (1H, d, *J* 7.6 Hz, ArH), 7.87 (1H, dd, *J* 1.8, 8.3 Hz, H-6), 8.09 (1H, s, H-4), 11.43 (1H, s, NHCO), 13.05 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.27 ($CH_2\underline{CH_3}$), 23.87 ($\underline{CH_2}CH_3$), 111.18 (CH, Ar), 113.71 (CH, Ar), 120.13 (CH, Ar), 121.73 (CH, Ar), 124.00 (C, Ar), 125.11 (CH, Ar), 128.10 (C, Ar), 128.22 (CH, Ar), 129.42 (C, Ar), 129.77 (CH, Ar), 130.82 (C, Ar), 140.08 (C, Ar), 143.76 (C=N), 164.54 (C=O), 167.87 (C=O). ν_{max} (solid)/(cm^{-1}) 3015

(md), 1737 (st), 1675 (md), 1617 (md), 1557 (md), 1454 (md), 1420 (md), 1365 (st), 1229 (st), 1216 (st), 1200 (st). MS *m/z* (**API-ES**): found 308 $(M-H)^-$ (100%). HRMS *m/z* (**API-ES**): found 308.1044 $(M-H)^-$, calculated for $C_{17}H_{14}N_3O_3$ 308.1035.

3-(Naphthalen-1-yl-hydrazono)-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid **(208e).** This was obtained as a orange solid (0.027 g, 0.081 mmol, 80%) from **206** (0.036 g, 0.103 mmol) and 1-naphthylhydrazine (0.020 g, 0.103 mmol) in a similar manner as described for preparation of **208a**, mp > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.04 (1H, d, *J* 8.4 Hz, H-7), 7.54-7.6 (5H, m, 5 x CH, Ar), 7.87-7.94 (3H, m, 3 x CH, Ar), 7.96 (1H, d, *J* 7.8 Hz, ArH), 7.94 (1H, s, H-4), 11.54 (1H, s, NHCO), 13.71 (1H, s, NNH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 109.55 (CH, Ar), 111.24 (CH, Ar), 119.61 (CH, Ar), 120.44 (CH, Ar), 121.57 (C, Ar), 122.32 (C, Ar), 123.72 (CH, Ar), 125.25 (C, Ar), 127.14 (C, Ar), 127.22 (CH, Ar), 127.27 (CH, Ar), 129.47 (CH, Ar), 129.53 (CH, Ar), 131.17 (CH, Ar), 134.44 (C, Ar), 137.44 (C, Ar), 144.06 (C=N), 164.80 (C=O), 167.83 (C=O). ν_{max} (solid)/(cm^{-1}) 3146 (md), 3117 (md), 1671 (st), 1614 (st), 1569 (st), 1402 (md), 1294 (md), 1259 (md), 1216 (md), 1198 (st), 782 (md), 763 (st). MS *m/z* (**API-ES**): found 332 $(M+H)^+$ (100%). HRMS *m/z* (**API-ES**): found 332.1015 $(M+H)^+$, calculated for $C_{19}H_{14}N_3O_3$ 332.1035.

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Appendix

Crystal data and structure refinement for compound 154.

Empirical formula	$C_{18}H_{16}N_2O_5$
Formula weight	340.33
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)/c
Unit cell dimensions	a = 28.02(3) Å, α= 90°.
	b = 8.328(7) Å, β= 92.414(17)°.
	c = 26.51(2) Å, γ = 90°.
Volume	6179(9) $Å^3$
Z	16
Density (calculated)	1.463 Mg/m^3
Absorption coefficient	0.108 mm^{-1}
F(000)	2848
Crystal size	0.60 x 0.25 x 0.15 mm^3
Theta range for data collection	0.73 to 25.00°.
Index ranges	-33<=h<=30, -8<=k<=9, -10<=l<=31
Reflections collected	14303
Independent reflections	10208 [R(int) = 0.0922]
Completeness to theta = 25.00°	93.9 %
Absorption correction	SADABS
Max. and min. transmission	1.000 and 0.560
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	10208 / 0 / 902
Goodness-of-fit on F^2	0.944
Final R indices [I>2sigma(I)]	R_1 = 0.1023, wR_2 = 0.2253
R indices (all data)	R_1 = 0.2377, wR_2 = 0.3025
Largest diff. peak and hole	0.416 and -0.394 e. $Å^{-3}$

Crystal data and structure refinement for compound 160.

Empirical formula	$C_{12}H_{13}NO_4$
Formula weight	235.23
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)/n
Unit cell dimensions	a = 3.8311(7) Å, α= 90°.
	b = 13.934(3) Å, β= 91.549(4)°.
	c = 21.340(4) Å, γ = 90°.
Volume	1138.8(4) $Å^3$
Z	4
Density (calculated)	1.372 Mg/m^3
Absorption coefficient	0.104 mm^{-1}
F(000)	496
Crystal size	0.30 x 0.20 x 0.10 mm^3
Theta range for data collection	1.75 to 25.13°.
Index ranges	-4<=h<=3, -16<=k<=14, -25<=l<=25
Reflections collected	5657
Independent reflections	2028 [R(int) = 0.0305]
Completeness to theta = 25.13°	99.2 %
Absorption correction	SADABS
Max. and min. transmission	1.000 and 0.668

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2028 / 0 / 154
Goodness-of-fit on F^2	1.063
Final R indices [I>2sigma(I)]	R_1 = 0.0469, wR_2 = 0.1144
R indices (all data)	R_1 = 0.0588, wR_2 = 0.1231
Largest diff. peak and hole	0.273 and -0.186 e. $Å^{-3}$

Crystal data and structure refinement for 144l.

Empirical formula	$C_{18}H_{18}N_2O_5$
Formula weight	342.34
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	Pbca
Unit cell dimensions	a = 15.070(2) Å, α= 90°.
	b = 10.2193(15) Å, β= 90°.
	c = 21.462(3) Å, γ = 90°.
Volume	3305.2(8) $Å^{-3}$
Z	8
Density (calculated)	1.376 Mg/m^3
Absorption coefficient	0.102 mm^{-1}
F(000)	1440
Crystal size	0.40 x 0.20 x 0.15 mm^3
Theta range for data collection	1.90 to 25.03°.
Index ranges	-17<=h<=17, -12<=k<=12, -23<=l<=25
Reflections collected	15775
Independent reflections	2919 [R(int) = 0.0705]
Completeness to theta = 25.03°	99.8 %
Absorption correction	SADABS
Max. and min. transmission	1.000 and 0.797
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2919 / 0 / 229
Goodness-of-fit on F^2	0.975
Final R indices [I>2sigma(I)]	R_1 = 0.0532, wR_2 = 0.1215
R indices (all data)	R_1 = 0.0792, wR_2 = 0.1344
Largest diff. peak and hole	0.219 and -0.202 e. $Å^{-3}$

Crystal data and structure refinement for 144e.

Empirical formula	$C_{18}H_{17}ClN_2O_5$
Formula weight	376.79
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	Pbca
Unit cell dimensions	a = 15.6372(18) Å, α= 90°.
	b = 10.4205(12) Å, β= 90°.
	c = 21.628(3) Å, γ = 90°.
Volume	3524.2(7) $Å^{-3}$
Z	8
Density (calculated)	1.420 Mg/m^3
Absorption coefficient	0.249 mm^{-1}
F(000)	1568
Crystal size	0.20 x 0.15 x 0.12 mm^3
Theta range for data collection	1.88 to 25.05°.
Index ranges	-18<=h<=16, -12<=k<=12, -25<=l<=25
Reflections collected	16750

Independent reflections	3123 [R(int) = 0.0367]
Completeness to theta = 25.05°	99.9 %
Absorption correction	SADABS
Max. and min. transmission	1.000 and 0.744
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3123 / 0 / 238
Goodness-of-fit on F^2	0.945
Final R indices [I>2sigma(I)]	R_1 = 0.0390, wR_2 = 0.0979
R indices (all data)	R_1 = 0.0483, wR_2 = 0.1037
Largest diff. peak and hole	0.307 and -0.180 e. $Å^{-3}$

Crystal data and structure refinement for 149a.

Empirical formula	$C_{19}H_{19}N_3O_7$
Formula weight	401.37
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)/c
Unit cell dimensions	a = 23.470(3) Å, α= 90°.
	b = 10.5529(15) Å, β= 98.418(3)°.
	c = 7.6841(11) Å, γ = 90°.
Volume	1882.7(5) $Å^{-3}$
Z	4
Density (calculated)	1.416 Mg/m^3
Absorption coefficient	0.110 mm^{-1}
F(000)	840
Crystal size	0.35 x 0.25 x 0.18 mm^3
Theta range for data collection	0.88 to 25.06°.
Index ranges	-27<=h<=24, -12<=k<=9, -9<=l<=8
Reflections collected	9156
Independent reflections	3333 [R(int) = 0.0393]
Completeness to theta = 25.06°	99.7 %
Absorption correction	SADABS
Max. and min. transmission	1.000 and 0.785
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3333 / 0 / 262
Goodness-of-fit on F^2	1.139
Final R indices [I>2sigma(I)]	R_1 = 0.0937, wR_2 = 0.2417
R indices (all data)	R_1 = 0.1211, wR_2 = 0.2596
Largest diff. peak and hole	0.665 and -0.316 e. $Å^{-3}$

Crystal data and structure refinement for 149b.

Empirical formula	$C_{19}H_{19}N_3O_7$
Formula weight	401.37
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)/n
Unit cell dimensions	a = 13.0842(15) Å, α= 90°.
	b = 8.4749(10) Å, β= 103.139(2)°.
	c = 17.211(2) Å, γ = 90°.
Volume	1858.5(4) $Å^{-3}$
Z	4
Density (calculated)	1.434 Mg/m^3
Absorption coefficient	0.111 mm^{-1}
F(000)	840

Crystal size	0.40 x 0.20 x 0.20 mm^3
Theta range for data collection	1.77 to 25.08°.
Index ranges	-15<=h<=7, -10<=k<=10, -20<=l<=20
Reflections collected	9104
Independent reflections	3287 [R(int) = 0.0273]
Completeness to theta = 25.08°	99.4 %
Absorption correction	SADABS
Max. and min. transmission	1.000 and 0.687
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3287 / 0 / 265
Goodness-of-fit on F^2	0.951
Final R indices [I>2sigma(I)]	R_1 = 0.0409, wR_2 = 0.1020
R indices (all data)	R_1 = 0.0482, wR_2 = 0.1072
Largest diff. peak and hole	0.297 and -0.236 e. $Å^{-3}$